MEMBRANE SEPARATION OF BIOACTIVE LYCOPENE FROM TOMATO JUICE

A Thesis

by

FELIPE ANDRES ARANA RODRIGUEZ

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2004

Major Subject: Food Science and Technology

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ABSTRACT

Membrane Separation of Bioactive Lycopene from Tomato Juice. (May 2004)
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Chair of Advisory Committee: Dr. Luis Cisneros-Zevallos

This thesis is a study of the concentration and purification of lycopene from tomatoes and tomato juice using membrane technology and solvent extraction.

In the first part of the study, three polymeric ultrafiltration (UF) membranes (PCI's FP 200, FP 100, and ES 404) were screened for lycopene concentration in a cross-flow pilot plant-scale membrane unit with tomato juice containing 10-11 mg lycopene/100g of tomato juice. PCI's FP 200 membrane was the best in terms of flux with average of 155 LMH at 60°C and 50 psi TMP for a 4.5 X concentration process. The tomato concentrate obtained as retentate, contained 51.7 mg lycopene/100g sample.

In the solvent extraction study, five solvents (ethanol, ethyl acetate, tetrahydrofuran, acetone, and isopropanol) were tested at ambient temperature as cosolvents with hexane for extraction of lycopene from the tomato concentrate obtained by UF. Ethanol:hexane (4:3) resulted in the highest recovery. Multiple extractions of the same tomato paste with fresh ethanol:hexane resulted in an all-trans-lycopene yield of 91% after 3 extractions at a solvent-to-solids ratio of 45 ml per g. Total recovery of carotenoids from the tomato concentrate was 58.8 mg carotenoids/100g tomato concentrate where all-trans-lycopene counted for 87.9%, β-carotene for 4.9%, 13-cis-lycopene for 3.2%, 7-cis-lycopene for 2.2%, and unidentified lycopene cis-isomers for 1.7%.

In the nanofiltration study, five polymeric membranes were screened with lycopene-hexane extracts. The DS 7 manufactured by Osmonics-Desal was the best in terms of flux, rejection and stability. The DS 7 membrane resulted in an average flux and rejection of 152 LMH and 72% respectively at 26.7°C and 400 psi when concentrating lycopene in hexane from 17.1 µg/ml to VCR 5. Preliminary design calculations indicate a 5-stage nanofiltration system can recover 90.2% of the lycopene and could result in a final retentate stream with 157 mg/l lycopene or more and a permeate stream with as low as 3.6 mg/l lycopene that can be recycled to the extraction stage. Economic calculations show that the industrial application of membrane technology for recovering lycopene is promising and profitable.

DEDICATION

A mi madre por todo su esfuerzo, sacrificio, cariño y amor para sacarnos adelante.

A Elsy por su apoyo, motivación, comprensión, paciencia y amor.

A mi hermana por su apoyo y constructivas críticas.

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1. INTRODUCTION

Lycopene is a naturally occurring pigment of the carotenoid family found in a variety of fruits and vegetables (e.g. tomatoes, guava, watermelon, papaya and grapefruit). Lycopene has been found to reduce the incidence of several types of cancers and cardiovascular diseases. Since the human body cannot synthesize it, lycopene must be supplied by food or nutritional supplements. Of the more than 50 dietary carotenoids, lycopene is the most prevalent in the Western diet and the most abundant in human serum (Nguyen and Schwartz 1999).

People are more aware of the health benefits of lycopene and as a result, supplements and functional foods containing this nutraceutical are increasingly popular. In 2000, the annual North America market for human antioxidants was about 800 million US$. One third of the American population regularly takes supplements and there is little doubt that with an aging and more educated population, this market will grow. Consumer awareness of benefits of antioxidants has grown from 14% awareness in 1992 to 57% in 1999 (Madley 2001).

Lycopene can be obtained by biosynthesis, chemical synthesis or by solvent extraction from natural sources such as fruits. Solvent extracted lycopene is used for human consumption and cosmetic use. Lycopene purification normally requires several steps which can include chromatography, adsorption and elution, filtration, precipitation, crystallization, evaporation, and/or drying. The current technology used for lycopene production and purification is energy intensive, renders low yields, and low product concentrations.

Because of its health benefits and the high cost of processing, pure lycopene and the nutraceuticals and functional foods containing it, are high price products. High purity lycopene (>95%) is sold at 80-100 US$ per milligram. Nutraceutical capsules of 15 mg lycopene (with other phytochemicals) are sold at 20 US$ per bottle of 30 capsules.

Membrane technology can offer an alternative to the industry in its search for lower operating cost and higher yields because is a proven low-energy, low temperature, and low-cost method of separation. The objective of this research was to develop methods using membrane technology for concentration and purification of lycopene extracted from tomato juice, which was chosen as a representative sample. Ultrafiltration (UF) was used to separate lycopene and other large solutes from the tomato juice. Lycopene was then extracted from the tomato

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concentrate using solvents. The lycopene-containing extract was concentrated and separated from the solvents using nanofiltration (NF). The solvents could be recycled back to the process. Operating conditions were mild enough to minimize damage to the lycopene.

The experimental approach for the project was as follows:

1. Membrane concentration of tomato juice
   - Initial screening of UF membranes based on flux and lycopene rejection.
   - Batch concentration of tomato juice at pilot plant scale using the best UF membrane.
2. Solvent extraction of lycopene from the tomato concentrate obtained in the membrane concentration.
   - Determination of the best solvent and optimum solvent/paste ratio.
   - Selection of the solvent with the best extraction efficiency and compatibility with the membrane material for the bench scale NF recovery.
3. Membrane recovery of lycopene from tomato extracts.
   - Screening of commercially available polymeric nanofiltration membranes for stability to the organic solvents, flux, and lycopene rejection.
   - Study on the flux and selectivity (rejection) of lycopene-organic solvent solutions for the best membrane as influenced by pressure, temperature, and volumetric concentration ratio.

Considering the health benefits that lycopene provides, valuable information was obtained so as to present membrane separation of lycopene as a promising processing technology for the retention of its biological potency and towards its use in the functional foods and nutraceutical industries.

2. LITERATURE REVIEW

2.1. Lycopene

Over the past decade, certain plant substances, which have come to be known as phytochemicals, have been the focus of attention because of their potential health benefits. Lycopene is one of such substances which belong to a broad class of lipophilic compounds referred to as carotenoids (Bruno and Wildman 2001).

Lycopene is the compound responsible for the red color in tomato fruit. It is found in higher concentration in tomatoes and tomato products, such as tomato sauces and ketchup, and in smaller amounts in other fruits such as guava, watermelon, papaya and grapefruit (Clinton 1998).

Of the more than 50 dietary carotenoids, lycopene is the most prevalent in the Western diet and the most abundant in human serum (Nguyen and Schwartz 1999).

2.1.1. Physical properties

Physical properties of lycopene are shown in Table 2.1.

Table 2.1- Physical properties of lycopene. (Shi and Le Maguer 2000).

Molecular formula	$C_{40}H_{56}$
Molecular weight	536.85 Daltons
Melting point	172 – 175 ^{O}C
Crystal form	Long red needles from a mixture of carbon disulphide and ethanol.
Powder form	Dark reddish-brown
Solubility	Soluble in chloroform, hexane, benzene, carbon disulphide, acetone, petroleum ether.
	Insoluble in water, ethanol, methanol.
Sensitivity	Light, oxygen, high temperature, acids.

2.1.2. Chemical structure and properties

Carotenoids can be divided into two groups. Carotenoid species in the first group are the highly unsaturated hydrocarbon carotenoids (or non-oxygenated) such as lycopene, α-carotene, β -carotene, γ-carotene, and ξ-carotene. In the second group are their oxygenated derivatives xanthophylls, for example, β-cryptoxanthin, lutein and zeaxanthin. The two groups of

carotenoids share common structural features such as polyisoprenoid structure and a series of centrally-located conjugated double bonds. The chemical structure of all-trans isomer lycopene molecules are shown in Figure 2.1.

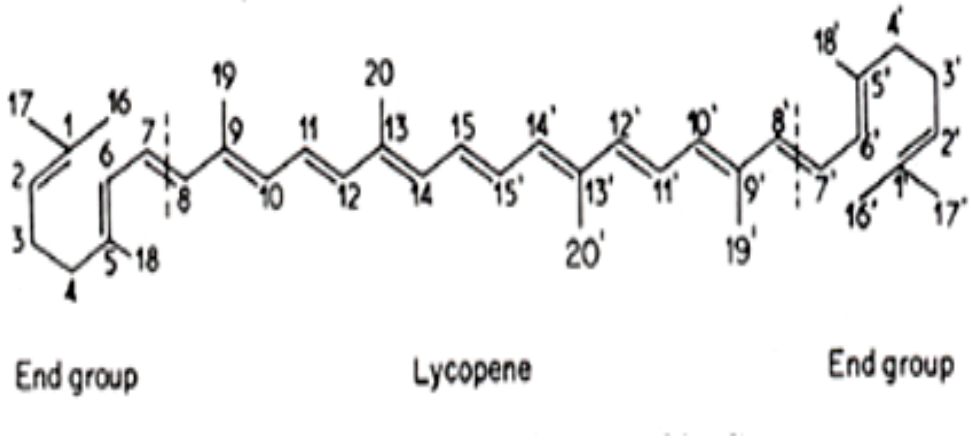

Figure 2.1- Molecular structure of all-trans isomers of lycopene. (Emenhiser and others 1995)

Lycopene is characterized by a symmetrical and acyclic open-chain unsaturated carotenoid with double bonds, from which 11 are conjugated double bonds arranged in a linear array (Fig. 2.1). The numbering of the molecule from the end to the center is 1 to 15, for the additional methyl groups it is 16 to 20, and for the symmetrical part it is 1' to 20'.

Color and antioxidant activities of lycopene are a consequence of its unique structure, an extended system of conjugated double bonds. The series of conjugated double bonds constitutes a chromophore which impart carotenoids their particular light absorption in the ultraviolet (UV) and visible region of the spectrum. Lycopene maximum absorption values are shown in Figure 2.2.

Being acyclic, lycopene possesses symmetrical planarity and has no vitamin A activity. As a highly conjugated polyene, it is particularly susceptible to oxidative degradation. Another type of change that lycopene undergoes readily as a polyene is cis-trans isomerization. With very few exceptions, lycopene from natural plant sources exist predominantly in the all-trans configuration, the most thermodynamically stable form (Zechmeister and others 1941; Wilberg

and Rodriguez-Amaya 1995; Emenhiser and others 1995). As a result of the 11 conjugated carbon-carbon double bonds in its backbone, lycopene can theoretically assume 2^{11} geometrical configurations. In fact, only about 72 lycopene cis-isomers are structurally favorable (Zechmeister 1962).

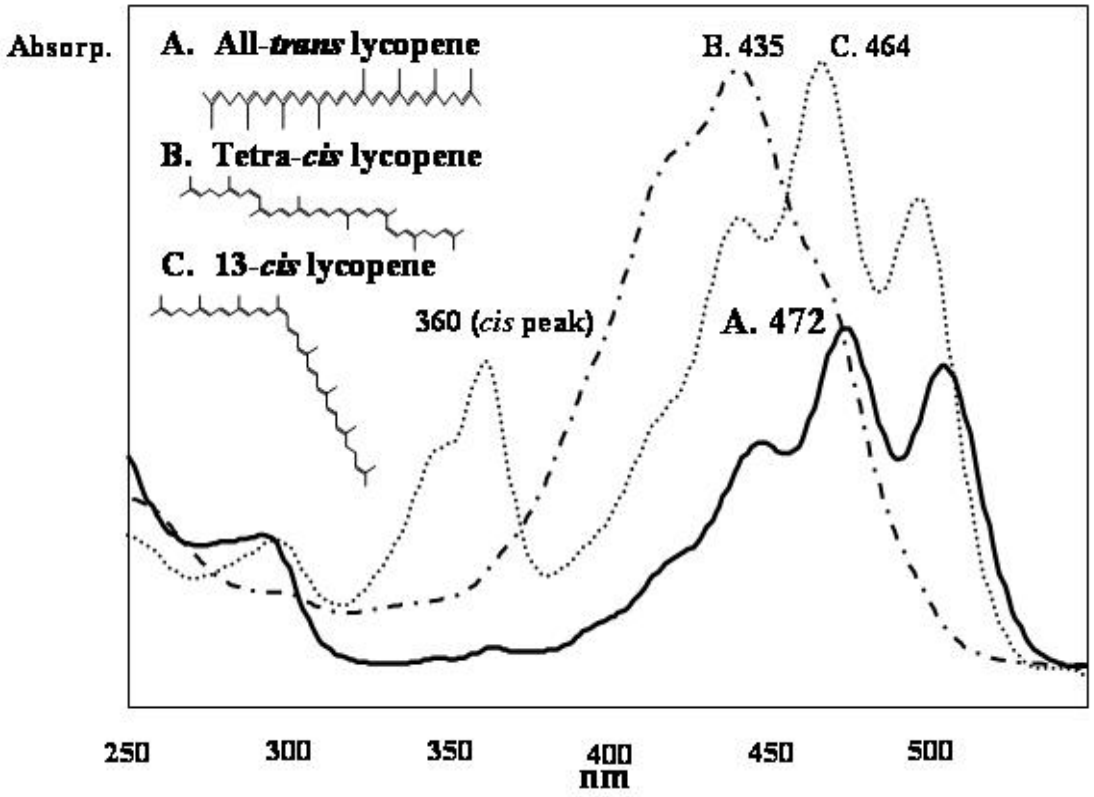

Figure 2.2 Absorption spectra of all trans- (──) and two cis- (- - -) lycopene. (Ishida and others 2001).

Cis-isomers of lycopene have distinct physical characteristics and chemical behaviors from their all-trans counterpart. Some of the differences observed as a result of a trans- to cis - isomerization reaction include decreased color intensity, lower melting points, smaller extinction coefficients, a shift in the lambda maximum, and the appearance of a new maximum in the ultraviolet spectrum (Zechmeister and Polgar 1944). The appearance of the new maxima in the

ultraviolet region so-called "cis-peaks," and their relative intensity are useful in assigning identification of lycopene isomers.

2.1.3. Biological properties

Lycopene exhibits many unique and distinct biological properties, including that as an antioxidant. Through their conjugated double bound system, they are able to quench efficiently the energy of deleterious forms of oxygen (singlet oxygen) and to scavenge a large spectrum of free radicals. Lycopene is among the most efficient singlet oxygen quenchers of the natural carotenoids (Di Mascio and others 1989; Conn and others 1991). There are considerable differences in the quenching rate constants (K_q) for various carotenoid species (Table 2.2).

2.1.4. Clinical implications

The majority of studies involving lycopene prior to the past two decades primarily focused on its physical and chemical properties in the context of color stability in food processing. There was a rise in interest to the biological effects of lycopene after the publication of a several key findings by Di Mascio and others (1989), Levy and others (1995), and Giovannucci and others (1995).

Table 2.2- Antioxidant activities of carotenoids.(Di Mascio and others 1989; Conn and others 1991, 1992; Miller and others 1996).

Lycopene	
(a) Singlet oxygen quenching, $K_q \times 10^9$ ($m^{-1}\ s^{-1}$)	31
(b) Radical scavenging (Trolox equivalents)	2.9
(c) Reaction of carotenoid radical anions with O_2 K x 10^8 ($m^{-1}\ s^{-1}$)	2.0
Other carotenoids' singlet oxygen quenching $K_q \times 10^9$ ($m^{-1}s^{-1}$)	
γ-carotene	25
α-carotene	19
β-carotene	14
Lutein	8
Astaxanthin	24
Bixin	14
Canthaxanthin	21
Zeaxanthin	10

Di Mascio and others (1989) discovered that lycopene is the most efficient singlet oxygen quencher of the biological carotenoids, including β-carotene. Levy and others (1995) showed lycopene to be a more potent inhibitor of human cancer cell proliferation than either α-carotene or β-carotene. Giovannucci and others (1995) confirmed these findings by the Harvard Health Professionals Follow-Up Study, in which the relationship between intake of various carotenoids, retinol, fruits, and vegetables and the reduced risk of prostate cancer was examined for 47,894 male subjects. These authors concluded that consumption of fresh tomatoes, tomato sauce, and pizza, which account for the bulk of dietary lycopene intake, is significantly related to a lower incidence of prostate cancer.

Many epidemiological and oncological studies suggest that carotenoid-rich diets help to maintain high levels of serum carotenoids and reduce the incidence of several types of cancer and cardiovascular diseases (Nguyen and Schwartz 1999). Accumulated human epidemiological evidence indicates that diets high in tomatoes may reduce the risk of developing cervical, colon, esophageal, rectal, and stomach cancers (Bjelke 1974; Cook-Mozaffari and others 1979; Tajima and Tominaga 1985; Batieha and others 1993; Ramon and others 1993; Potischman and others 1994; Franceschi and others 1994).

Lycopene's ability to act as an antioxidant and scavenger of free radicals that are often associated with carcinogenesis is potentially a key to the mechanism for its beneficial effects on human health (Khachik and others 1995). Lycopene may prevent carcinogenesis and atherogenesis by interfering passively with oxidative damage to DNA and lipoproteins (Gester 1997; Clinton 1998). It may also inhibit the formation of LDL cholesterol's oxidized products, which, in turn, have been suggested to participate in the early stages of coronary heart disease (Ojima and others 1993; Diaz and others 1997; Weisburger 1998). Lycopene's protective effects against oxidative stress are also illustrated when human skin is irradiated with UV light – lycopene was found to be preferentially destroyed relative to β-carotene, suggesting either a more active or a more-effective role (Ribaya-Mercado and others 1995).

Despite the overwhelming evidence linking lycopene to various beneficial bioactivities, a number of inconsistencies exist in the epidemiological data regarding lycopene's role in disease prevention. For example, Steinmetz and others (1993) found no association between lung cancer risk and either tomatoes or three carotenoid-rich food groups. Likewise, Jarvinen and others (1997) found that lycopene intake was not significantly related to the occurrence of breast cancer in a Finnish prospective cohort study. Differences in the oxidative environment of the

lung compared to other cancer sites and the uniqueness of breast cancer carcinogenesis have been cited as key factors influencing lycopene's effectiveness in these cases. These findings, nonetheless, suggest that further research on lycopene is needed not only to discover lycopene's mode of action but also to understand the scope of its effectiveness (Nguyen and Schwartz 1999).

2.1.5. Natural occurrence of lycopene

Lycopene is found predominantly in the chromoplast of plant tissues. Since lycopene and other carotenoids are photosynthesized by plants and microorganisms, they constitute the main source of all animal carotenoids. One of the functions of lycopene and related carotenoid species is to absorb light during photosynthesis, thereby protecting plants against photosensitization. Sometimes the brilliant colors of lycopene are masked by the green chlorophyllic pigments (i.e., in green vegetables and leaves). In a number of cases, the chlorophyll content decreases as plants mature, leaving the lycopene and other carotenoids responsible for the bright colors of most fruits (pineapple, orange, lemon, grapefruit, strawberry, tomato, paprika, rosehip) and many flowers (eschscholtzia, narcissus). Lycopene and other carotenoids also contribute to the colors of some birds (flamingo and canary), insects, and marine animals (shrimp, lobster and salmon) (Shi and Le Maguer 2000). Concentration of lycopene in different fruits and vegetables is shown in Table 2.3.

From the carotenoids occurring in ripe tomatoes, lycopene is the last to form and its formation increases especially after the breaker stage (color change from green to pink) of the berry. Lycopene is the most abundant carotenoid in ripe tomatoes, comprising approximately 80 – 90 % of those pigments present. Regarding the distribution of lycopene and other carotenoids in tomato, the skin and the pericarp of the fruit are particularly rich (D'Souza and others 1992; Sharma and Le Maguer 1996). The concentration of lycopene in tomato skin is about 3-5 times higher than in whole mature tomatoes (Shi and Le Maguer 2000).

2.1.6. Stability during food processing

Most stability studies on lycopene in food systems concern degradation. Lycopene, as a conjugated polyene, may be expected to undergo at least two changes during tomato processing, isomerization and oxidation. The isomerization of lycopene has shown to take place both in tomato products and in pure lycopene forms, and can take place during processing. On the other

Table 2.3- Lycopene content of fruits and vegetables, and tomato products. (Beerh and Siddappa 1959; Gross 1987, 1991; Mangels and others 1993; Nguyen and Schwartz 1999).

MATERIAL	lycopene content (mg / 100 g wet basis)
Fresh tomato fruit	0.72 - 20
Watermelon	2.3 - 7.2
Guava (pink)	5.23 - 5.50
Grapefruit (pink)	0.35 - 3.36
Papaya	0.11 - 5.3
Rosehip puree	0.68 - 0.71
Carrot	0.65 - 0.78
Pumpkin	0.38 - 0.46
Sweet potato	0.02 - 0.11
Apple pulp	0.11 - 0.18
Apricot	0.01 - 0.05
Tomato soup	3.99
Tomato juice	7.83
Tomato salsa	9.28
Tomato paste	30.07
Pizza sauce (from pizza)	32.89

hand, the conversion of cis-isomer to trans-form is another reaction, which can occur during the product storage. Cis-isomers are in the unstable state, whereas the trans-isomers are in the stable ground state (Shi and Le Maguer 2000).

Cole and Kapur (1957a, 1957b) reported significant losses of lycopene in serum-free tomato pulp samples following thermal treatment at 100 ^{o}C in the presence of oxygen, with or without light. The intensity of illumination and temperature were found to be in direct correlation with lycopene degradation in the presence of oxygen. Sharma and Le Maguer (1996) reported the kinetics of lycopene degradation in tomato pulp solids to be a pseudo first-order reaction. Boskovic and others (1979) observed a reduction of all-trans lycopene content by up to 20 % following processing and extended storage of dehydrated tomato products. In other lycopene-containing fruits, such as papaya slices, food processing operations, such freezing and canning, led to a significant decrease in total carotenoid content, of which lycopene is a major component (Cano and others 1996).

In contrast, a number of studies on the thermal stability of carotenoids in processed fruits and vegetables have found that hydrocarbon carotenoids such as lycopene, α-carotene, β-carotene are relatively heat-resistant. According to Khachik and others (1992a, 1992b), most of these carotenoids remain stable following bench-top food preparation. Common heat treatments during food preparation such as microwaving, boiling, steaming, and stewing did not significantly alter the carotenoid distribution in green vegetables and tomatoes. Other studies have also reported the levels of lycopene cis- isomers in thermally processed tomato products to be low (Clinton and others 1996; Emenhiser and others 1996). Nguyen and Schwartz (1998) have confirmed that in tomato products of varying moisture content, fat content, and container type, thermal treatments during usual food preparation or commercial production processes do not result in significant losses of lycopene or a shift in the distribution of cis-lycopene isomers. Thus, lycopene in the diet from both fresh and processed foods is consumed predominantly as the all-trans configuration. The elevated levels of cis-isomers observed in human biological samples cannot be attributed to consumption of thermally processed food but rather to in-vivo mechanism which are still unclear.

It is difficult to reconcile the differences in these findings, since a number of factors may account for the changes in lycopene levels. The inability to distinguish between chemical degradation and geometrical isomerization, for example, is a common limitation (Nguyen and Schwartz 1999). Lycopene is more stable in native tomato fruit tissues and matrices than in isolated or purified form (Simpson and others 1976), as a result of the protective effects of cellular constituents such as water. Therefore, care must be exercised to minimize the loss of lycopene through oxidation or isomerization during extraction, storage, handling, and analysis to accurately account for these cause and-effect changes (Nguyen and Schwartz 1999).

2.1.7. Effect of processing on lycopene bioavailability

In addition to knowing the amount of lycopene present in a food, it is important to know the bioavailability with respect to the absorption in the human body. Bioavailability is defined as the fraction of an ingested nutrient that is available to the body through absorption for utilization in normal physiological functions and for metabolic processes (Jackson 1997). The US FDA definition of bioavailability of a drug is “the rate and extent to which the active substances or therapeutic moiety is absorbed from a drug product and becomes available at the site for action” (Benet and Shiner 1985). The concept of bioavailability of a nutrient or nutraceutical has close

relationship with the estimate of bioavailability of pharmaceutical compounds (Shi and Le Maguer 2000).

2.1.7.1. Effect of trans and cis- isomer forms

All trans-isomers are the predominant species in tomatoes and tomato products. In various tomato-based foods, the all-trans isomer is comprised of 35-96 % of total lycopene The proportion of 5-cis-isomer in tomato-based foods is 4-27%, with considerably lower amounts of other isomers (Schierle and others 1997). Isomers 15-cis, 13-cis, 11-cis, 9-cis, 7-cis, 5-cis are found in human serum. The 5-cis-isomer of lycopene has been identified in various tomato-based foods and human tissues by NMR spectroscopy (Zumbrunn and others 1985). The cis-isomers of lycopene contribute more than 50 % to total lycopene in human serum and tissue (Krinsky and others 1990).

The literature is not clear about which lycopene isomer has the highest bioavailability. One view is that the trans- form has a higher bioactivity and conventional processing methods convert it to the cis form which reduces lycopene bioavailability. (Khachik and others 1992b; Emenhiser and others 1995; Shi and Le Maguer 2000; Wilberg and Rodriguez-Amaya 1995). Others concluded that food processing is in fact a value-added step, since more lycopene becomes bioavailable following thermal treatment. Stahl and Sies (1992, 1996) stated that heating of tomato juice results in an improvement in uptake of lycopene in humans and that the cis-isomers (5-cis, 9-cis, 13-cis, 15-cis) are better absorbed than the all-trans form by the human body. Boileau and others (1999) stated that cis-isomers of lycopene are more bioavailable that trans-form probably because cis-isomers are more soluble in bile acid micelles and may be preferentially incorporated into chylomicrons. Schierle and others (1997) concluded that cis-isomers are less likely to crystallize, more efficiently solubilized in lipophilic solutions, and more readily transported within cells or in the tissue matrix. The concentration of cis-isomers can be increased by processing. Heat treatment promotes isomerization of lycopene in foods, from trans- to cis-isomeric form. The degree of isomerization is directly correlated with the intensity and duration of heat processing (Shierle and others 1997).

2.1.7.2. Effect of food matrices

Cooking or fine grinding of foods could increase the bioavailability of lycopene by disrupting or softening plant cell walls and disrupting lycopene-protein complexes (Hussein and

El-Tohamy 1990). Giovannucci and others (1995) compared the differences in lycopene bioavailability from fresh tomatoes with processed tomato products, and found the lycopene serum concentration was greater when consuming heat-processed tomato-based foods than unprocessed tomatoes. Gartner and others (1997) found that lycopene bioavailability from paste and processed tomato juice was significantly higher than from unprocessed fresh tomatoes. Thermal processing such as cooking and mechanical texture disruption such as chopping are convenient ways to enhance bioavailability by breaking down sturdy cell wall structures, disrupting chromoplast membranes, and reducing cellular integrity, thus making lycopene more accessible (Shi and Le Maguer 2000).

2.1.7.3. Effect of oil medium

Lycopene bioavailability from tomato-based food is significantly higher than from fresh tomatoes when co-ingested with oil. Thermal treatment and an oil medium are required to extract lycopene into lipophilic phase (Stahl and Sies 1992). When lycopene solubilizes in a lipophilic matrix, it is considerably reactive and more available; therefore, it could suitably perform its antioxidant activity (Shi and Le Maguer 2000).

2.1.7.4. Enhancement of lycopene bioavailability

Lycopene plasma levels increased significantly in human serum when processed juice was consumed, as compared to unprocessed tomato juice. The bound-form of lycopene in tomatoes is converted by processing temperatures, which makes it more easily absorbable by the body. These results suggest that food processing may improve the availability of lycopene in tomato-based foods for absorption. Gartner and others (1997) pointed out that heat treatment can improve the bioavailability of lycopene without significantly changing the cis-isomer composition of the heat-treated foods. Stahl and Sies (1992) reported that heat treatment of tomato juice at 100 ^{o}C for 1 h resulted in 20-30 % cis-isomers in the serum of humans who drank the juice.

People are more aware of the health benefits of lycopene and as a result, supplements and functional foods containing this nutraceutical are increasingly popular. In 2000, the annual North America market for human antioxidants was about 800 US$ million. One third of the American population regularly takes supplements and there is little doubt, that with an aging and

more educated population, this market will grow. Consumer awareness of benefits of antioxidants has grown from 14% awareness in 1992 to 57% in 1999 (Madley 2001).

2.1.8. Lycopene production and patents

Industrial production of lycopene is in high demand by pharmaceutical companies and for functional food development. Although there are no specific publications about lycopene manufacturing, recent patents show the methods and processes that can be used in its production. Lycopene can be produced basically by three routes: chemical synthesis, solvent extraction from natural sources, and biosynthesis.

2.1.8.1. Production by chemical synthesis

The production of lycopene as a high-purity compound has been linked in the past to chemical synthesis. For example, US patent No. 2,842,599 describes the synthesis of lycopene by condensing 2,6,11,15-tetramethyl-2,4,6,8,10,12,14-hexadecaheptaene-1,16-dial with triphenyl-(3,7-dimethyl-2,6-octadien-1-ylidene)-phosphine. Schulz and others (1978), in US patent 4,105,855 assigned to BASF, describe the synthesis of lycopene by dimerizing 3,7,11,15-tetramethylhexadeca-2,4,6,8,10,14-hexaen-1-yl-triphenylphosphonium bisulfate. Meyer (1992), in US patents 5,166,445 and 5,208,381 assigned to Hoffman-La Roche, also describes chemical synthesis of lycopene and intermediary phosphonium salts.

2.1.8.2. Production by solvent extraction of natural sources

The preparation of crystalline lycopene of high purity from fruit and vegetable generally requires initial extraction with organic solvents, and then various steps of purification such as chromatography, adsorption and elution, filtration, precipitation, crystallization, evaporation, and/or dried. Bombardelli and others (1999), in US patent 5,897,866, describe production of oleoresin containing 4.7 % of lycopene using tomatoes or tomato skins and seeds as starting materials. A simplified block diagram of the process is shown in Figure 2.3. Israeli Patent No. 107999, filed Dec. 23, 1993, has proposed a process for lycopene similar to a one currently used for the extraction of paprika. In this process, pulp obtained after the separation of waste materials and tomato serum is extracted with hexane to provide tomato oleoresin. Then, oleoresin containing about 2-10% of lycopene is further processed to obtain a free-flowing powder containing about 40-100 % Lycopene.

Bortlik and others (2001), in the European patent EP 1,103,579 assigned to Nestle, describes a process for extraction and recovery of lycopene from tomato, tomato pomace, pink grapefruit, watermelon, guava and papaya using boiling ethanol in consecutive washing and extraction steps. Yields higher than 50% and concentrations up to 1700μg/g dry material are reached. Block diagrams are shown in Figure 2.4. Atsushi and others (2002), in Japanese patent JP 2002125619 assigned to Kikkoman, claim to provide a Lycopene extract with high lycopene content (≥ 9 % wt. on anhydrous basis) and perfectly free from other residual solvent than ethanol which is used in the production process. This lycopene composition is obtained by subjecting tomato treated product to centrifugation, micro-filtration, ethanol fractionation, dried and pulverized. Additionally, the lycopene rich powder is washed with 30 – 100 vol. % ethanol and finally heated for solvent evaporation. Blocks diagram in Figure 2.5.

Methods using solvents have the disadvantage that the final product can retain traces of them. Also, there are chemical and physical hazards during processing and in waste streams. A variation of the above processes is described by Ausich and Sanders (1999) in US patent 5,858,700 where Lycopene crystals with 75-95% purity are obtained from tomato oleoresin using saponification of various triglycerides and phosphonates at high temperature followed by dilution with water. Block diagrams shown in Figure 2.6. Hartal and others (1999), in US patent 5,965,183, describe the preparation of lycopene crystalline concentrates stabilized in a food-compatible liquid medium which lycopene is insoluble, such as ethylene glycol or glycerol. Starting with tomato oleoresin, lycopene crystals are suspended in the liquid medium by grinding them between 1-3 μm.

2.1.8.3. Production by biosynthesis

Lycopene can be obtained from a micro-algae or from natural biosynthesis of certain fungi. Nonomura (1987), in patents US 4,713,398 and US 4,680,314, and Rose and others (1995), in US patent 5,378,369, describe various methods for carotenoids extraction from a micro-algae of the Dylaniella type. Estrella del Castro and others (2002), in the European patent EP 1201762 assigned to Vitatene (Spain), describe production of crystalline lycopene (purity 90% - 98 %) starting from a natural biosynthesis source and employing solvents that are regarded as natural or toxicologically harmless (Generally Recognized As Safe, GRAS). Stages of the process are shown in Figure 2.7.

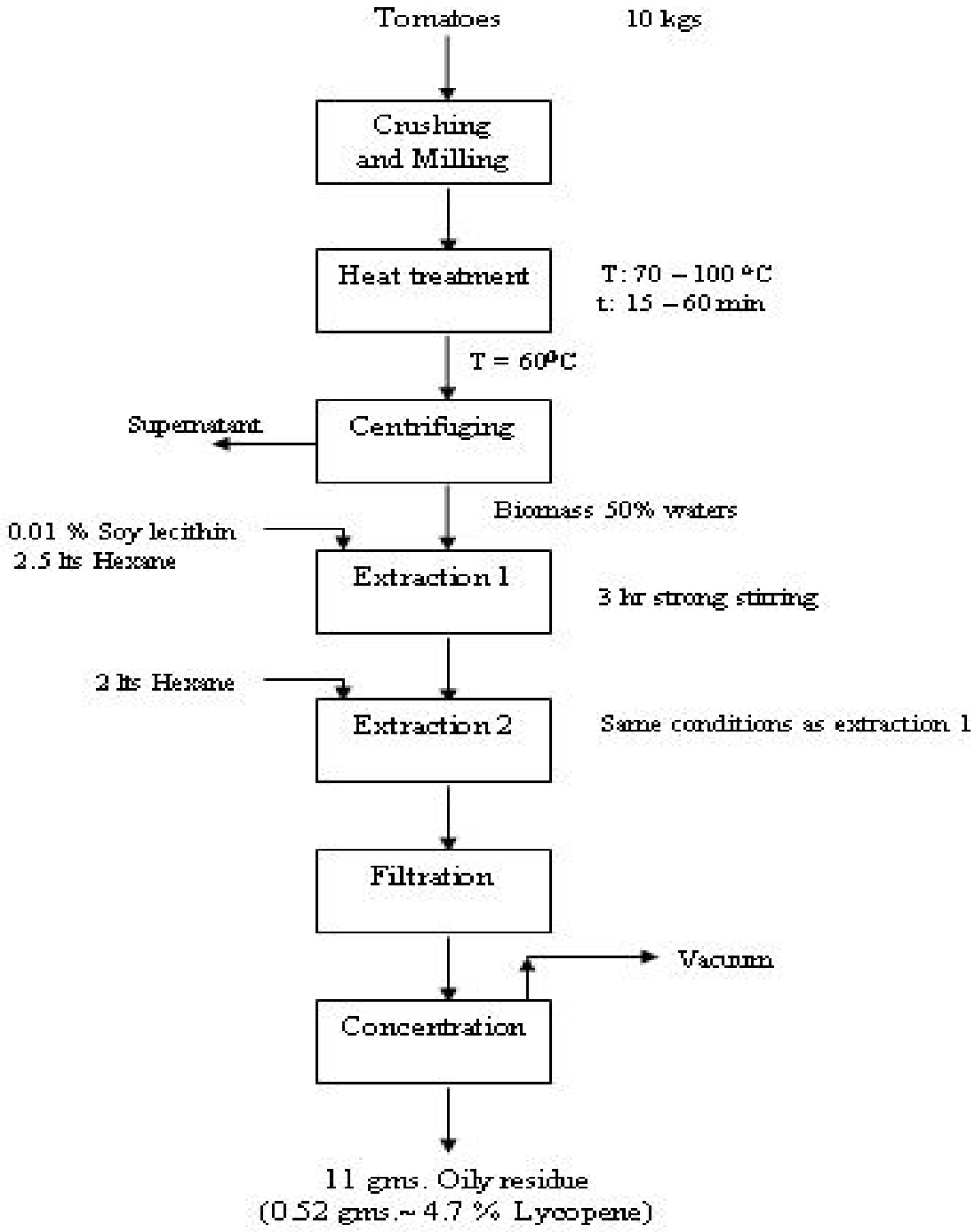

Figure 2.3 Lycopene process of Bombardelli and others (1999). US patent 5,897,866.

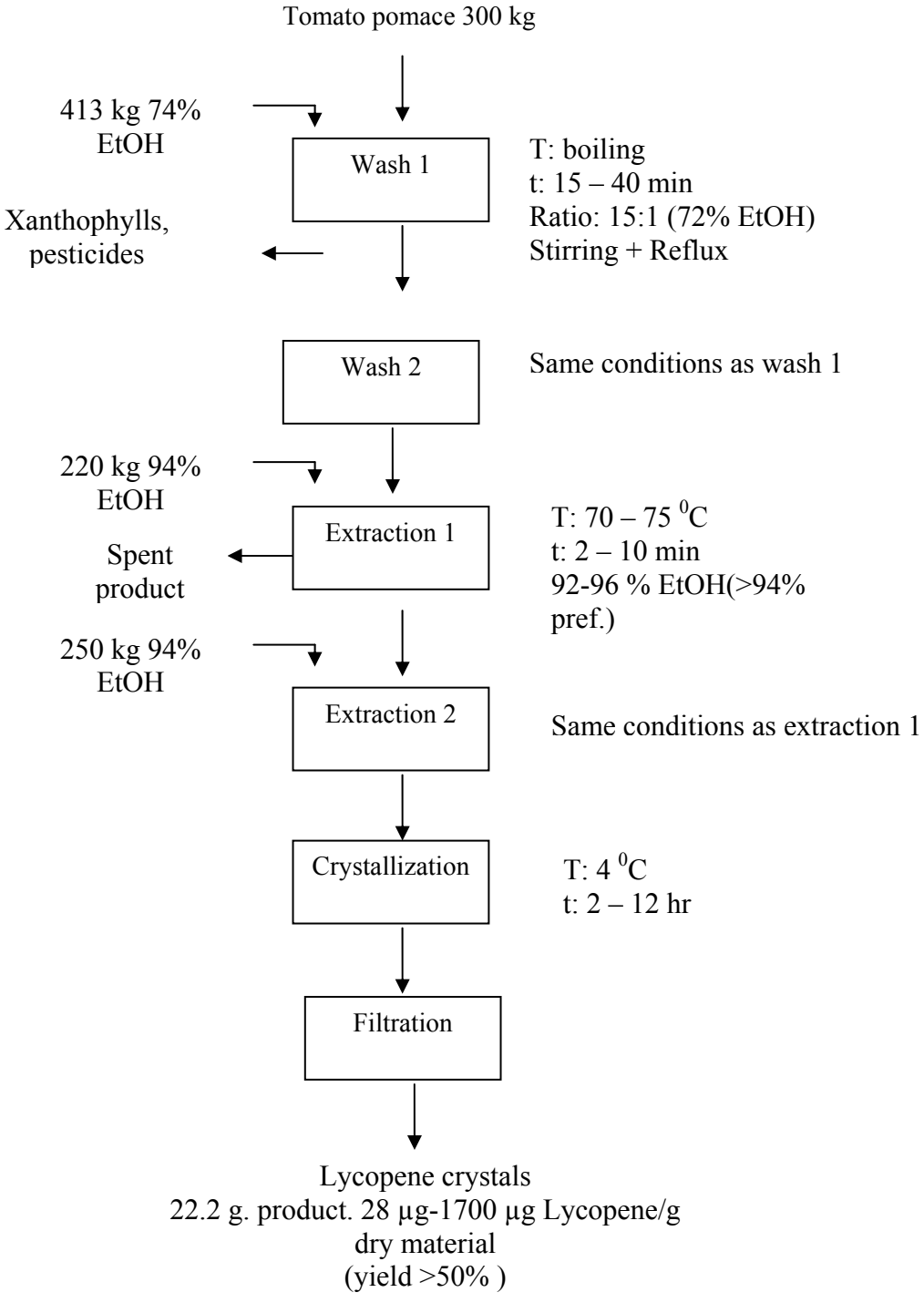

Figure 2.4 Lycopene process of Bortlik and others (2001). European Patent EP 103579.

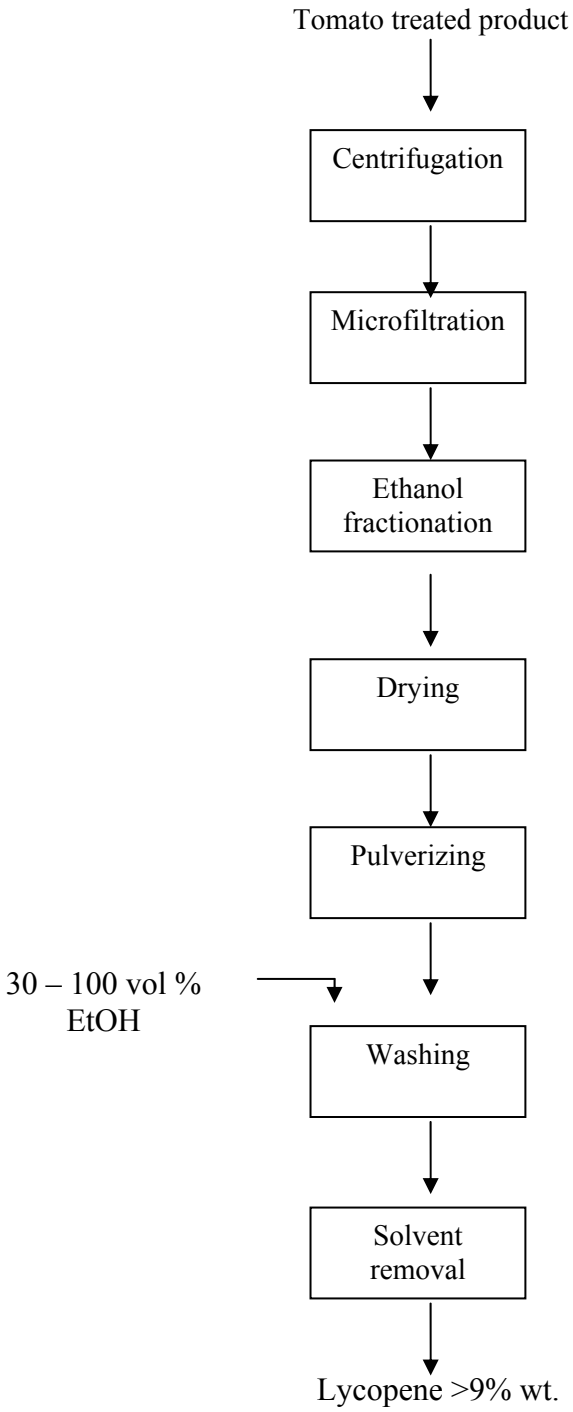

Figure 2.5 Lycopene process of Atsushi and others (2002). Japanese patent JP 2002125619.

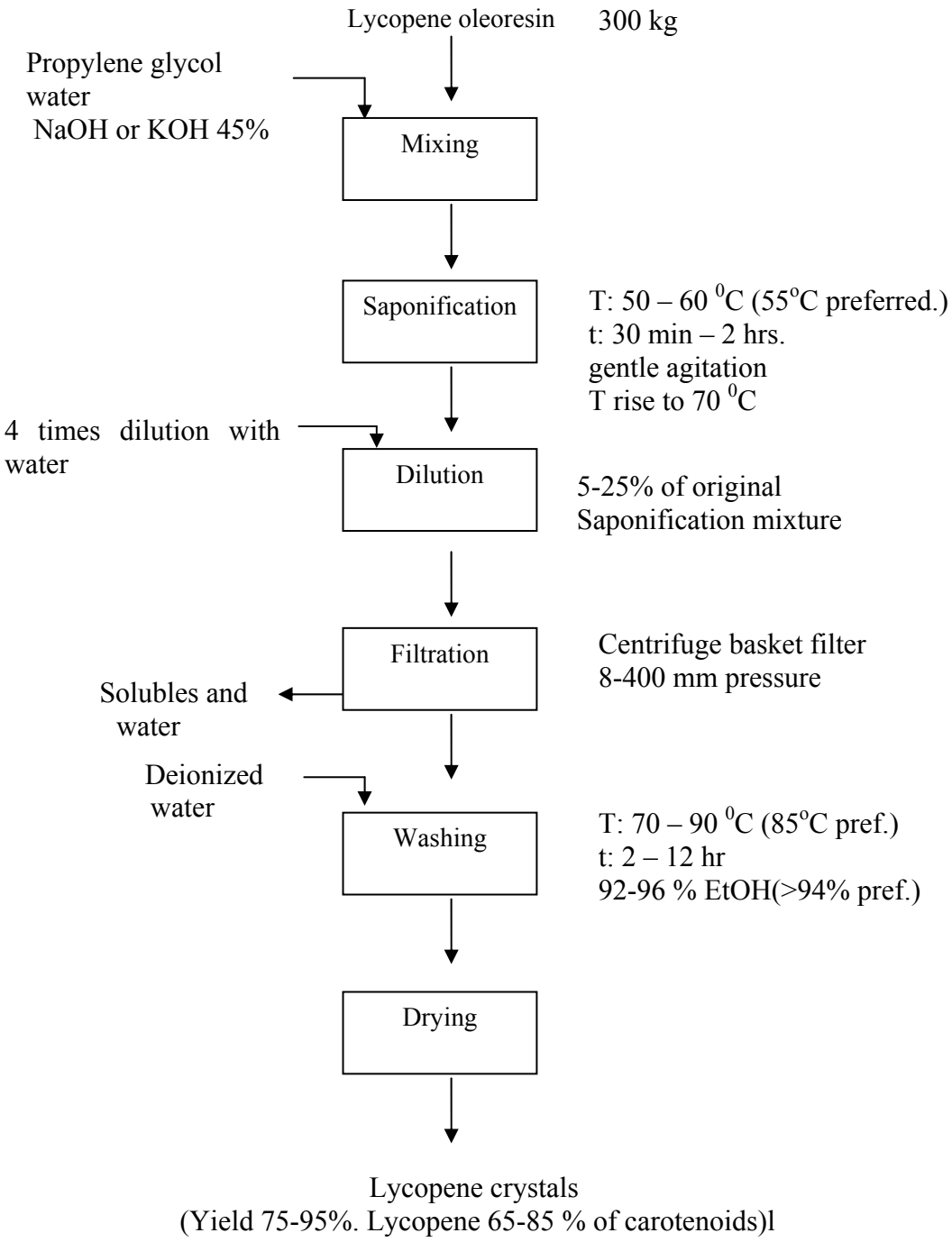

Figure 2.6 Lycopene process of Ausich and others (1999).
US Patent 5,858,700.

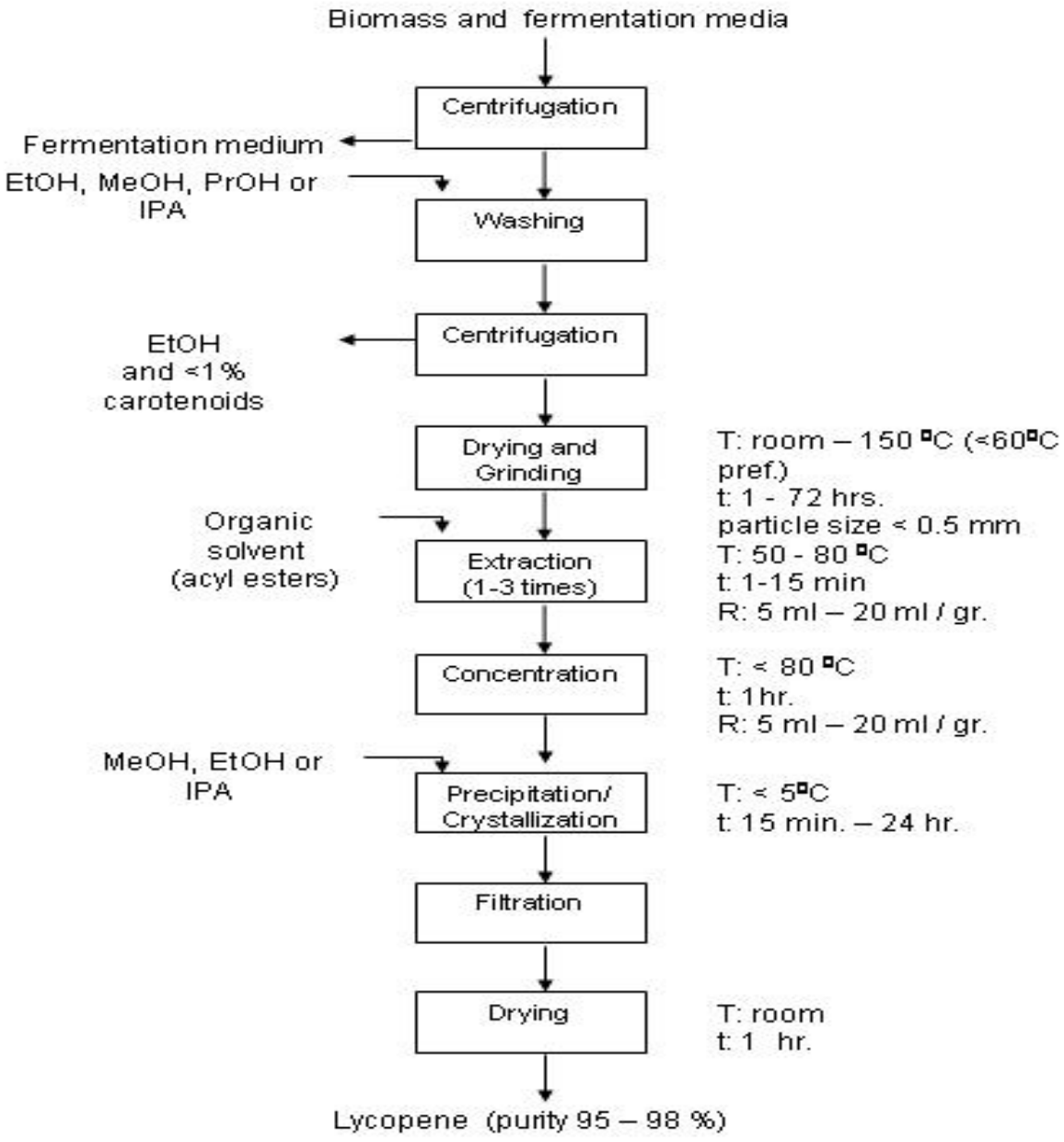

Figure 2.7 Lycopene process of Estrella del Castro and others (2002). European patent EP 1201762.

2.2. HPLC analysis of lycopene

Lycopene is usually extracted with solvents such as chloroform, hexane, acetone, benzene, petroleum ether, ethanol, or carbon disulphide, prior to chemical analysis for quantitative determination. Exposure of extracted lycopene to light should be avoided, and only gold, yellow, or red lights should be used (Landers and Olson 1986). Antioxidants such as butylated hydroxytoluene (BHT) should be employed in extraction and separation solvents to control oxidation and isomerization reactions of lycopene (Nguyen and Schwartz 1998). In addition, nitrogen or argon headspace can be employed to keep exposure to atmospheric oxygen to a minimum. A saponification step is the most effective method of removing unwanted lipids, chlorophylls, and other impurities after lycopene extraction. This procedure does not affect lycopene because lycopene is generally alkali-stable (Kimura and others 1990).

High-performance liquid chromatography (HPLC) is the conventional separation method of choice for lycopene. In general, lycopene is separated from other carotenoids using reverse-phase C_{18} columns. HPLC parameters of some selected methods using C_{18} columns are shown in table 2.4. Variations in the properties of the silica packing material in terms of particle size, porosity, carbon load, en-capping technique, and polymerization can greatly influence the sensitivity and selectivity of lycopene analysis (Sander and Wise 1987; Craft 1992; Epler and others 1992; Sander and others 1994).

To separate lycopene isomers, however, reversed-phase C_{30} stationary phase is often employed to achieve superior selectivity of isomers compared to C_{18} reversed-phase and silica normal-phase columns (Sander and others 1994; Emenhiser and others 1995). Not only the polymerically synthesized C_{30} columns provide excellent separation of the all-trans lycopene isomers from the cis counterpart, but they also exhibit remarkable selectivity among the individual cis isomers themselves (Emenhiser and others 1995; Rouseff and others 1996). Recently, another HPLC method using multiple columns in series has also been shown to comparably resolve cis and trans lycopene isomers (Shierle and others 1997). HPLC parameters of some selected methods using C_{30} columns are showed in Table 2.5.

Some rapid, efficient extraction methods for lycopene analysis have been developed involving microwave solvent extraction, and pressurized accelerated solvent extraction technologies in which the lycopene recoveries from samples ranged from 98 – 99.6 % (Benthin and others 1999).

Table 2.4- Reverse phase HPLC for lycopene and carotenoids determination using C18 columns.

REFERENCE	EXTRACTION	HPLC COLUMN	MOBILE PHASE, PARAMETERS
Wei and others 2001	2g crude tomato paste extract percoled with 5x50 ml Chloroform/MetOH (2:1) and dried with N2	Supelcosil ODS ((250x4.6 mm i.d.)	MetOH:Acetonitrile:Chloroform (47:50:6). Flow rate 1.0 ml/min, isocratic, 30 C, time 20 min. UV 472 nm
Tonucci and others 1995	Tomato puree in 500 ml tetrahydrofuran (10% of tomato puree)	Stainless steel Microsorb-MV C18 (250x4.6 mm i.d.) with Brownlee C18 guard column	Acetonitrile:MetOH:Methylene chloride:Hexane (40:20:20:20). Flow rate 0.7 ml/min, UV at 450 nm
Hart and others 1994	10 g ground sample homogenized, washed and filtered with 4x50 ml THF:MetOH and 3x50 ml Petroleum ether	polymeric C18 Vydac 201 TP54 (250x4.6 mm i.d.; 5 um 300 A)	Acetonitrile:MetOH:DCM (75:20:5), Flow rate 1.5 ml/min, isocratic, 22.5 C, time 50 min. UV at 450 nm
Hakala and Heinonen 1994	10 g tomato puree +300 ml petroleum ether	µ Bondapak C18 (10µm, 150x19mm, i.d.)	Acetonitrile:dichloromethane:MetOH (45:10:45), Flow rate 2.0 ml/min., isocratic, time 15 min. UV at 470 nm
Stahl and Sies 1992	5 ml tomato juice in 200 ml hexane-dichlomethane solution (5:1)	5 µm RP 18 endcapped column (4x250 mm)	MetOH:Acetonitrile:Dichloromethane: Water (7:7:2:0.16), flow rate 1.0 ml/min. UV at 460 nm.
Khachik and others 1992	50 g tomato puree in 500 ml tetrahydrofuran	Stainless steel (250 x 4.6 mm i.d.) Microsorb C18 (5 µm spherical particles) column	Acetonitrile:MetOH:Dichloromethane: Hexane (45:10:22.5:22.5), flow rate 0.7 ml/min. UV at 455, 470 nm.
Sadler and others 1990	4 g tomato puree with 100 ml hexane/ethanol/acetone (50:25:25)	Analytichem C18 (5µ) column (250x4.6 mm) with a Supelguard LC-18 guard column	MetOH:THF: Water (67:27:6), flow rate 2 ml/min, isocratic, run time 25 min, UV at 475 nm

Table 2.5- Reverse phase HPLC for lycopene and carotenoids determination using C30 columns.

REFERENCE	EXTRACTION	HPLC COLUMN	MOBILE PHASE, PARAMETERS
Ishida and others 2001	1-10 g tomato fruit homogenized with 20 ml MetOH, 0.01% ethylenediamine tetraacetic acid and BHA. Then filtered and washed with 2x20ml Dichloromethane. Final dried.	polyeric C30 (250x4.6 mm i.d.; 3 um) YMC	MTBE:MetOH:EthylAcetate (4:50:10). Flow rate 1.0 ml/min, isocratic, 28 C, time 30 min.
Bohm 2001	Standard solutions prepared with Hoffmann-La Roche carotenoids	polymeric C30 (250x4.6 mm i.d.; 5 um 200 A) YMC	MTBE (A) MetOH (B). Flow rate 1.3 ml/min, 60 % A 40 % B X 11 min. Then a 4 min linear gradient to 10 % A, 23 C, time 60 min.
Ferruzi and others 2001	5 g raw diced tomato homogenized with 25 ml MetOH and extracted with 3x25 ml Acetone/hexane (1:1)	polymeric C30 (250x4.6 mm i.d.; 3 um) YMC	MTBE:MetOH:Ammonium Acetate 1.0 M (6.5:41.5:2), Flow rate 1.0 ml/min, isocratic, 25 C, time 50 min.
Shi and others 1999	10 g tomato puree in 100 ml hexane/acetone/ethanol solution (2:1:1)	polymeric C30 (250x4.6 mm i.d.; 3 um)	MetOH:MTBE (62:38). Flow rate 1.0 ml/min, isocratic. UV 460 nm.
Nguyen and Schwartz 1998	10 g puree mixed with 50 ml methanol, 1 g CaCO3, and 3.0 g Celite, then extracted with acetone/hexane sln (1:1), saponified with 30% KOH for 60 min.	polymeric C30 (250x4.6 mm i.d.; 3 um)	MTBE:MetOH (40:60). Flow rate 1.0 ml/min, isocratic. UV at 200-800 nm.
Lessin and others 1997	30 g puree, 30 ml deionized water, 1 g calcium carbonate, 1g Celite, 25 ml methanol, homogenized and filtered, then extraction in 25 ml methanol, filter cake in 50 ml of acetone/hexane (50:50), repeated	polymeric C30 (250x4.6 mm i.d.; 5 um)	MetOH:MTBE (89:11). Flow rate 1.0 ml/min, isocratic. UV at 410 nm.
Clinton and others 1996	Tomato sample containing 2.5 ml of distilled water and ethanol (containing 2% butylated hydroxytoluene) was extracted by addition of 5 ml of 10% NaOH in methanol (30 min. at 60 C)	polymeric C30 (250x4.6 mm i.d.; 3 um) YMC	MTBE:MetOH (38:62). Flow rate 1.0 ml/min, isocratic. UV at 460 nm.

2.3. Membrane technology: basic concepts

Membrane separations have been applied widely in the food industry for purification and concentration. The major advantages of membrane separation are the ambient temperature of operation and no phase change or state of the food materials, which results in lower operating costs and less damage to thermally unstable products. A membrane basically acts as a barrier which permits passage of certain components while retaining other components of a mixture (Cheryan 1998). The semi-permeable media or membrane acts as a surface filter to split the feed stream into two effluents: a purified stream (permeate) and a stream more concentrated in solutes too large to pass through the pores of the particular membrane (retentate). The openings in the membrane material (pores) are so small that a significant fluid pressure is required to drive the liquid through them; the pressure required varies inversely with the size of the pores. There are four commonly accepted categories or "classes" of membranes, based on the particle size or molecular weight of the material they will remove from the carrier liquid. Microfiltration (MF) encompasses the separation of macro molecules from 200,000 to 1 million molecular weight (MW). Ultrafiltration (UF) with molecules 30,000 to 300,000 MW, nanofiltration (NF) with molecules 70 to 15,000 MW, and reverse osmosis (RO) with ions and molecules up to 600 MW (Paulson 1995).

The separation efficiency of a specific membrane is characterized by two main concepts: permeate flux and rejection rate. Permeate flux is defined as the permeation rate per unit of membrane area. Thus maximizing the flux will reduce size and cost. Permeate flux can be calculated as: Flux = Total quantity passed through membrane / (membrane area x time) (Cheryan 1998). Rejection rate measures the ability of the membrane to retain a certain molecule. The observed solute rejection rate R_i for a given specie i is given by $R_i = 1 - C_{ip} / C_{ir}$ (Kulcarni and others 1992), where C_{ip} the concentration in the permeate while C_{ir} is the corresponding value in the retentate. The volumetric concentration ratio (VCR) indicates how many fold the feed has been concentrated, and is defined as $VCR = V_f / V_r$ (Kulcarni and others 1992), where V_f and V_r are the volumes of feed and retentate, respectively.

Most membranes are organic (polymers), but some inorganic membranes have become available. Organic membranes are based on polysulfone, cellulose acetate, polyamide, polyacrylonitrile, fluoroplymers and other compounds. Inorganic membranes common materials are Alumina, Zirconia, Stainless steel, Carbon composite, and Silica. Depending on the application, various membrane designs are used, such as flat sheet, disc tube, hollow fiber, spiral

wound, and ceramic (Bemberis and Neeky 1986). Module design has a measurable effect on the hydrodynamic performance of the cross flow membrane device.

While most UF/NF applications are aqueous, researches also have indicated the feasibility of organic phase separations. The compatibility of membranes with different inorganic and organic solvents and feedstock varies with the membrane material. The most common UF / NF process configurations are batch concentration, feed and bleed, and diafiltration. The simplest batch concentration configuration is most commonly used in laboratory-scale and pilot-scale units (Cheryan 1998). The retentate is returned to the feed tank through the module. It is the fastest method of concentrating a specific amount of material. Another process design with total recycling of both retentate and permeate is often used in the study of membrane fouling, in which the flux decrease resulting from the increase of feed concentration can be ignored.

Other considerations for membrane separation are chemical and mechanical stabilities, and membrane material cost.

2.3.1. Applications with organic solvents

Membrane separation processes such as reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF) and microfiltration (MF) have widely been used for aqueous streams for over four decades. Their applications include seawater desalination, fruit juice clarification, dairy product concentration, waste water treatment, medical and blood applications and gas separations (Cheryan 1998). However, their use for non-aqueous separations has been limited. The few non-aqueous applications discussed in the literature usually deal with streams containing organic compounds with concentrations only up to a few thousand ppm such as oily waste streams and cleaning solvents (Cheryan and Rajagopalan 1998). There are very few examples of membrane applications with feeds containing 50-100% organic solvents. One example where solvent-stable membranes would be useful is the vegetable oil industry for degumming (Köseoğlu and others 1990; Iwama 1987), deacidification (Kale and others 1999; Raman and others 1996b; Snape and Nakajima 1996) and solvent recovery (Cheryan 1998; Raman and others 1996b). Other potential applications include solute concentration and fractionation in a variety of industries such as the food, pharmaceutical, biotechnology and petrochemical industries.

Some of these industries may have a large portion of their process streams in the form of organic solvents. In other cases, the organic solvent may be in trace amounts, usually in

industrial effluents that require treatment before disposal. Conventionally, these separations are carried out by thermal processes such as distillation and evaporation which are highly energy intensive. Membrane technology uses substantially less energy compared to these processes. There have been a few studies (Bhanushali and others 2001; Bitter and others 1989; Farnand and others 1984; Koops and others 2001; Köseoğlu and others 1990; Lencki and Williams 1995; Machado and others 1999; Musale and Kumar 2000; Raman and others 1996a, 1996b; Shukla and Cheryan 2002; Yang and others 2001) on evaluation of commercial and prototype membranes for stability in organic solvents in terms of pure solvent permeation, swelling or for separation of solutes in organic solvents. The general conclusion was that very few membranes are stable in commonly used industrial organic solvents such as alcohols, ketones or hydrocarbons. The use of NF membranes for pharmaceutical industries and the separation characteristics of the membranes have been studied (Sheth and others 2002; Whu and others 1999, 2000). For refinery process, White and Nitsch (2000) developed a polyimide membrane for solvent recovery from lube oil filtrates. Solvent resistant NF membranes are also applied in fine chemical synthesis for recycling of phase-transfer catalysts (Luthra and others 2001). However, the widespread use of membranes for non-aqueous separations has been hindered due to unavailability of commercial membranes that can perform the desired separations, have the desired stability and be low in cost. There have been efforts to develop solvent resistant membranes (Koenhen and Tinnemans 1992; Kumar and Musale 2000; White and Nitsch 2000; Zwijnenberg and others 1999). However, they are not commercially available. Currently, the only commercially available polymeric NF membranes proven to be stable in certain organic solvents are the MPF series from Kiryat-Weizmann (now part of Koch Membrane Systems) (Machado and others 1999; Nunes and Peinemann 2001; Raman and others 1996a; Sheth and others 2002; Whu and others 2000).

2.3.1.1. Membrane stability

The stability of membranes in organic solvents depends on the physicochemical characteristics of both solvents and membranes. Solvent interactions with membranes may result in swelling, plasticization or dissolution of membrane material and subsequent loosening of the membrane structure, leading to loss of mechanical strength under pressure. Some of the studies cited above also reported that the solvent characteristics such as molar volume, solubility parameter, viscosity, surface tension and dielectric constant and membrane characteristics such

as hydrophobicity, dielectric constant and solubility parameter play an important role in membrane stability. Hestekin and others (1999) observed a decrease in flux and rejection as ethanol concentration increases for negatively charged Desal HL membrane by Osmonics. They concluded that the higher the concentration of solvent , the lower the charge potential of the membranes and the solutes leading to a decline in rejection. Bhanushali and others (2001), Hestekin and others (2001), and Shukla and Cheryan (2002) demonstrated that flux has a linear relationship with inverse viscosity for NF and UF membranes at lower alcohol concentration when convective transport is dominant. At high alcohol concentration (>80 wt %), the pore dehydration phenomenon becomes dominant (Hestekin and others 2001). Bhanushali and others (2001) also extended this flux model to molar volume, surface energy and sorption values for non-aqueous system. Machado and others (1999) found that there was no correlation between flux and solvent molecular volume of organic solvents, but only solvent viscosity and surface tension of the membrane determine solvent permeation.

For long-term membrane performance in organic solvents, the only studies found were done by Shukla (2000) and White and Nitsch (2000). Shukla (2000) performed long-term stability test for three UF membranes. Membranes were preserved in 70% aqueous ethanol at ambient condition for up to 10 weeks. The flux and rejection tested at 60psi were stable only for the m-PAN membrane. The two regenerated cellulose membranes gradually fouled through the 10-week period. White and Nitsch (2000) developed a polyimide NF membrane and demonstrated excellent chemical resistance and viable flux and rejection after 2 months of continuous high pressure tests at 600 psi.

2.3.1.2. Characterization

Almost all NF membranes are composite in nature, with a UF membrane serving as the support for another polymer that acts as the perm-selective layer. It is possible that differential swelling of each layer could result in premature failure of the membrane. Koops and others (2001) and Yang and others (2001) showed that rejections of the same solute are significantly different for a given membrane when measured in solvents with different polarities. The implication of this finding is that the MWCO/pore size data provided by membrane manufacturers (which are usually measured in aqueous environments) may not be valid when used in organic solvents. There is little agreement in the literature on the mechanisms of solute-solvent-membrane interactions in organic solvents.

2.3.1.3. Conditioning

Many hydrophilic membranes can be made more resistant to solvent degradation by drying the membranes and subsequently soaking in a no aqueous solvent. However, the membrane's internal structure could collapse by intra-chain hydrogen bonding if dried. Many membranes sold commercially are pre-swelled in glycerol or similar humectants such that the membrane can be handled in a dry state. This is usually accomplished by treating membranes with a 10% solution of glycerol. After drying, the glycerol is left behind and maintains the pore structure through strong hydrogen bonding (Schonfelder and Wilke 1971). In order to use these membranes with organic solvents, it is important to provide appropriate "conditioning" to the polymer matrix. It is a general rule that the smaller the pores on a membrane, the more important it is to keep it submerged in the liquid (which is usually water) in order to maintain its porosity and flux. Abrupt substitution of water with non-aqueous liquids is very traumatic for highly dense membranes, such as reverse osmosis, NF and tight (low MWCO) UF membranes. The non-aqueous solvent usually has a lower surface tension and thus, according to Laplace's law, membranes will require higher pressures for replacing the interstitial water. To condition a hydrophilic membrane with a water-miscible organic solvent, the membrane has to be soaked in a series of successive baths of solvents, using a procedure first described by Van Oss (1970). The process essentially involves permeating the membrane, under pressure, sequentially with a series of solvents of decreasing polarity where the first solvent is miscible with water as well as the next succeeding solvent in the series, and so on. For example, to replace interstitial water with vegetable oil in a hydrophilic membrane, the membrane is submerged for at least one hour in successive baths with varying compositions: 30% water-70% ethanol; 5% water-45% ethanol-50% butanol; 100% butanol (renewed 3 times); 70% butanol-30% oil; 30% butanol-70% oil; 100% oil.

The importance of pressure in conditioning membranes was emphasized by Hafez and Koenitzer (1985), who permeated the treating solvents (C_1-C_4 alcohols/acetone) in succession to convert hydrophilic, water-containing cellulose acetate membranes. With denser membranes (RO, NF), low pressures coupled with short exposure times may not provide complete solvent exchange and could disrupt the polymer matrix. Conditioning is not required for all membranes. It is usually governed by the method of manufacture and casting of the polymer matrix. For instance, Wernick (1987) discovered that by preparing membranes in solvents based on

solubility parameter considerations, cellulose acetate membranes may be developed which require no solvent exchange for use with ketone-oil systems. The choice of solvents when the membranes are manufactured is based on the solubility parameter model (Klein and Smith 1972). According to this theory, every solvent is assigned three parameters, which represent the tendency of the solvent molecules to interact with one another or with other molecules via certain mechanisms. Similarly, each polymer has a solubility parameter, which defines its characteristic solubility region. The polymer is soluble in all solvents whose solubility parameters lie with this region and is insoluble in solvents outside the region (Klein and Smith 1972).

Consequently, a membrane classified as "solvent stable" should not be used with the entire range of organic solvents available but only with those that lie outside the solubility region of the polymers used to make the membrane (this includes the separating layer as well as the bottom support matrix). This concept has been utilized to develop novel polymers for solvent stable applications. For instance, Zschocke and Strathmann (1980) used poly-p-phenyleneterephthalamide, an aromatic polyamide that is soluble only in concentrated sulfuric acid. This would be appropriate for most no aqueous applications. Other approaches have included graft co-polymerization of the membrane layer and cross linking with gluteraldehyde (Musale and Kumar 2000). Similarly, interfacially polymerized membranes, which were developed in the 1970s for water desalination, have been marketed as solvent stable or compatible with a variety of organic solvents. These membranes are composed of highly cross-linked, and generally insoluble, condensation polymers that are formed in situ on a microporous film. Most of these membranes are formed by reacting di- or polyamines with multifunctional isocyanates or chlorides on an ultrafiltration membrane (usually polysulfone) as the support. However, the material used for support in most commercial membranes is not particularly stable to organic solvents. Some attempts have been made using a solvent stable support but these membranes presented poor fluxes (Black 1991).

3. SCOPE AND OUTLINE OF PROJECT

The overall objective of the project is to demonstrate the feasibility of extracting lycopene from fruits and vegetables, using solvents generally recognized as safe and membrane technology.

Current extractions processes of lycopene typically result in low concentrations of the bioactive compound in the solvent. This means that considerable amount of energy has to be spent to evaporate the solvent and recondense it for reuse in the plant. This increases the cost of lycopene manufacturing and increases the handling of volatile solvents. We propose to use an alternate method involving membrane technology for the concentration and purification of lycopene and also to aid in solvent recycling. This work focuses on the concentration and purification of lycopene from tomato juice using ultrafiltration (UF), solvent extraction, and nanofiltration (NF). This membrane separation process can be used to obtain lycopene from tomato, tomato juice, tomato pomace (seeds and skins), watermelon, guava or any fruit and vegetable containing this important bioactive compound, and can be integrated in existing lycopene plants that use solvent extraction and conventional methods of purification. This approach is shown in Figure 3.1 and is described below:

1. Membrane concentration of tomato juice.
   - Initial screening of UF membranes based on flux and lycopene rejection.
   - Batch concentration of tomato juice at a pilot plant scale using the best UF membrane.
2. Solvent extraction of lycopene from the tomato paste obtained in the membrane concentration.
   - Determination of the best solvent and optimum solvent/paste ratio.
   - Selection of the solvent with the best extraction efficiency and compatibility with the membrane material for the bench scale NF recovery.
3. Membrane recovery of lycopene from tomato extracts

The initial step was to evaluate and screen commercial NF membranes in hexane for stability and flux. Based on these studies with pure solvents, a few membranes were selected for more detailed studies with lycopene solutions to optimize NF parameters. The best NF membrane was chosen to purify and concentrate lycopene from the tomato extract and to recover

the solvent for recycle. The major measured parameters were flux, rejection of lycopene, and yield of lycopene.

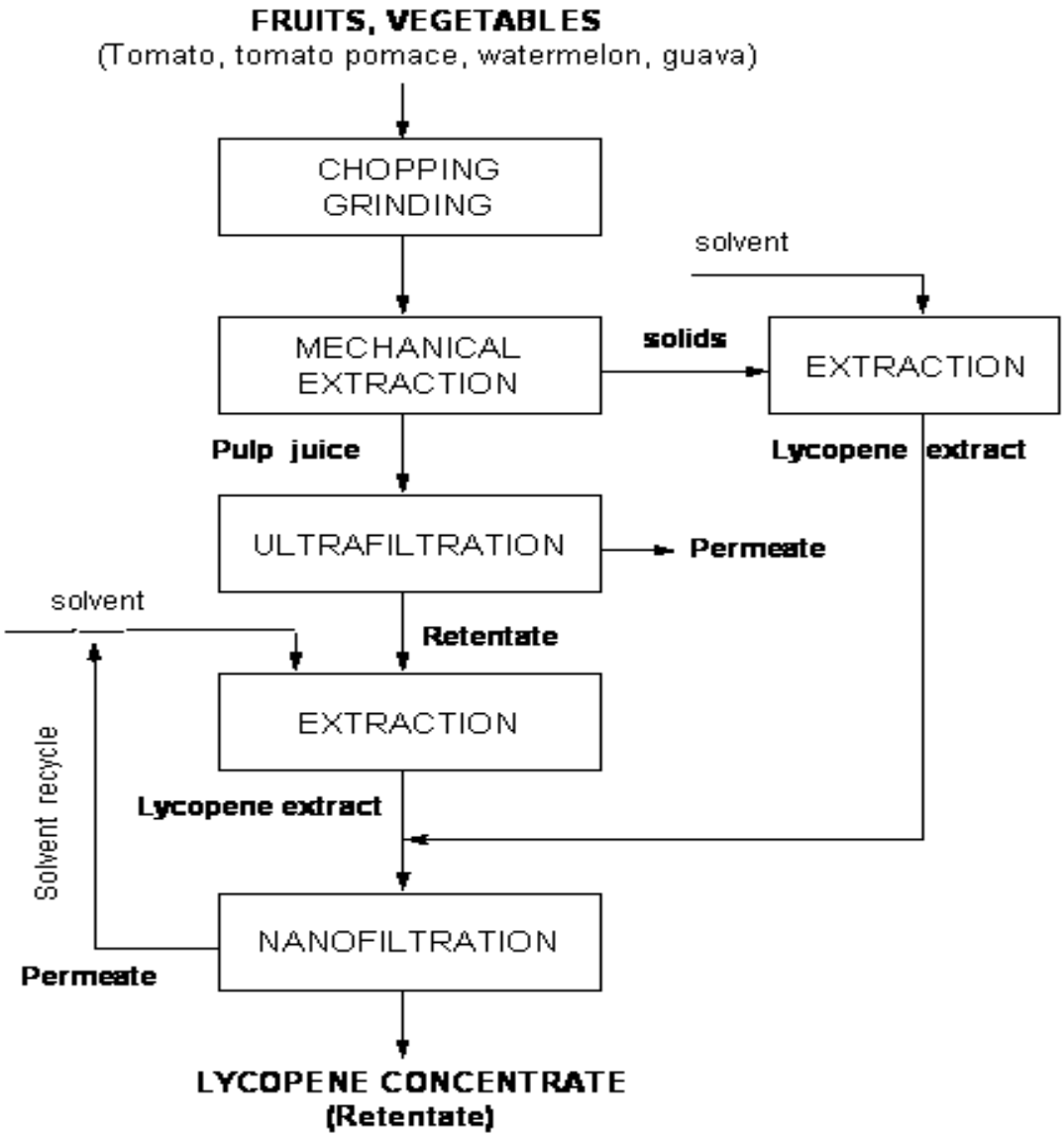

Figure 3.1 Proposed process for producing lycopene from fruits and vegetables.

4. MEMBRANE CONCENTRATION OF TOMATO JUICE

4.1. Introduction

Concentration of tomato juice presents a difficult problem because it has a high pulp content (25% fiber) and a high viscosity (which behaves in a non-newtonian manner). The rheological properties of the juice are affected by the method of juice manufacture. The break temperature, which is the temperature which the tomatoes are exposed after chopping, can give different levels of enzyme inactivation which, in turn, affects final viscosity. A cold break (at 65°C) results in less inactivation of the natural enzymes, which results in high viscosity. Thus, membrane concentration of tomato juice is limited by both the osmotic pressure and the viscosity. If the fibers were separated, the remaining serum would behave as a water-like liquid (Cheryan and Alvarez 1995).

The usual concentration of natural tomato juice is 4.5-5 °Brix. Commercial tomato sauces are 8-12 °Brix, and tomato pastes are 28-29 °Brix. Because of the fiber content and particle size, tubular modules are probably best; for example little fouling has been reported with the reverse osmosis (RO) PCI AFC-99 tubular membrane (Pepper and others 1985; Merlo and others 1986). In addition, flux was unaffected by the feed velocity in the range 0.3-4 m/s. this has been attributed to a “tubular pinch” effect, e.g., the solids are suspended in the middle of the flow channel, and thus away from the wall where the membrane is located (Watanabe and others 1982). Higher fluxes by UF compared with microfiltation (MF) membranes were reported for tomato juice concentration. Smaller pore size in the UF membrane avoided fouling with the tomato juice solids.

The objective of this study was to increase lycopene content in the tomato juice using membrane technology to minimize volumes prior to the solvent extraction and nanofiltration separation. Comparison between tomato juice fluxes and rejections for three polymeric membranes (PCI ES 404, PCI FP 100, and FP 200) was analyzed. Batch concentration of tomato juice at a pilot plant scale using the best UF membrane (PCI FP 200) was performed.

4.2. Materials and methods

Separation experiments were carried out with a UF pilot plant-scale unit (Fig. 4.1). The unit could be used up to 1000 psig and temperatures up to 177°C. A tubular cross-flow PCI

membrane cell which could accommodate one to six tubular membranes was used. The effective surface area was 0.0479 m^2 per tube.

Transmembrane pressures (TMP) were 50 psig for FP 100 and FP 200 membranes and 100 psig for ES 404. The flow and TMP are set manually by means of a gear type speed reducer and valves installed at the inlet and outlet of the membrane cell. The retentate is recycled to the feed tank and permeate can be recycled as well. Samples can be taken at any time in the experiment. The temperature was 60°C. A heat exchanger with supply of water and steam was used to keep the temperature stable. Flux and rejection values are an average of at least three measurements with the same membrane tube; flux measurements were carried out three times for different membranes of the same type. Schematic of the set up is shown in Figure 4.2.

The membranes used were PCI FP 100, FP 200, and ES 404 obtained from PCI Membrane Systems Inc. (Milford, OH, USA). The information provided by the manufacturer is summarized in Table 4.1. All membranes, except PCI ES-404, were rated as UF membranes. The pretreatment of membranes was as follow: The tube was rinsed to remove the preservatives and humectants recirculating deionized (DI) water into the membrane system. Then, the membrane was cleaned for 15 to 20 minutes with a sodium hydroxide solution 15% at ambient temperature and pH 9-11. The membrane was then pressurized in the unit at minimum TMP and DI water was allowed permeating through the membrane to wash off any preservatives and/or caustic solution in the pores. This cleaning and pressure testing was usually done a few times until the water flux was reproducible.

Name-brand and store-brand canned tomato juice were purchased in local stores and stored at 5 °C until processed. Three 35 L juice runs, one for each membrane, were performed. Permeate and retentate flows were measured each 15 minutes once the operating pressure was reached. Ultrafiltration was continued until the inlet pressure could be maintained opening the back pressure valve (maximum feed concentration). The same procedure was repeated to obtain triplicates of the experiment. Permeate and retentate samples were stored at -80°C until lycopene determination was performed.

Lycopene content was determined by high performance liquid chromatography (HPLC). Sample extraction for analysis was based in method described by Taungbodhitham and others (1998). Briefly, 2 g sample was extracted in plastic Falcon tubes with an Ultraturrax homogenizer at 16,000 rpm for 3 minutes using 35 ml ethanol:hexane (4:3). Extract was filtrated under reduced pressure in a Buchner filter and collected in a separation funnel. Residue was re-

Figure 4.1 Ultrafiltration membrane unit used in experiment.

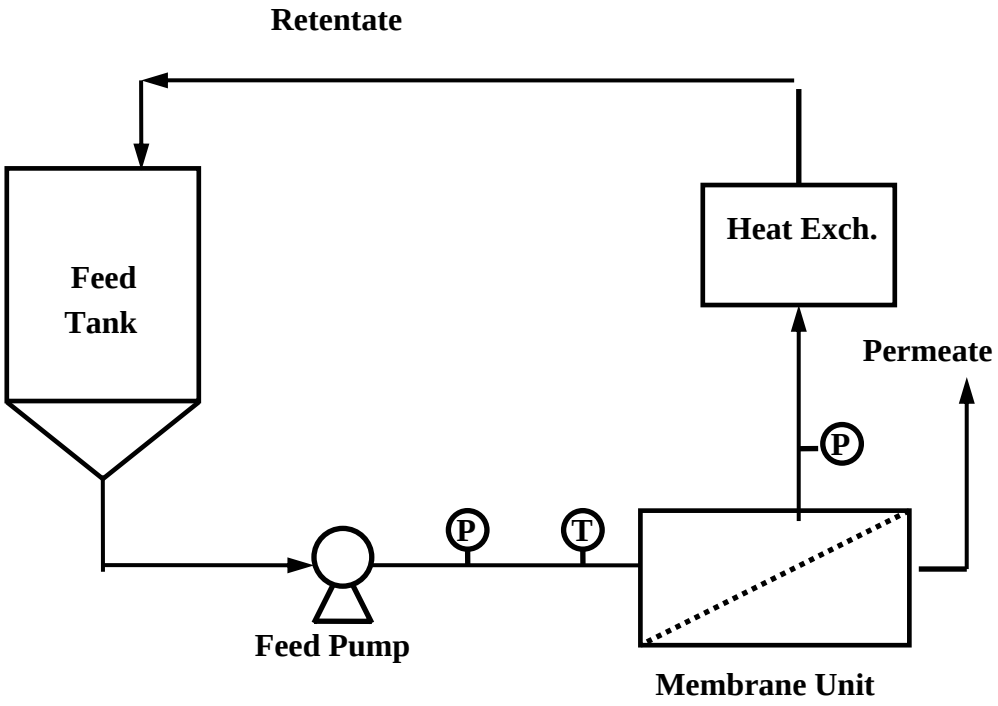

Figure 4.2 Schematic of batch pilot plant-scale ultrafiltration unit.

Table 4.1- Ultrafiltration membrane characteristics as specified by manufacturer.

MEMBRANE	PCI FP 200	PCI FP 100	PCI ES-404
TYPE	Ultrafiltration Hydrophilic	Ultrafiltration Hydrophilic	Nanofiltration Hydrophilic
MATERIAL	PVDF	PVDF	Polyethersulphone
SOLVENT RESISTANCE			
Alcohols	Good	Good	Limited Stability
Hexane	Limited Stability	Limited Stability	Limited Stability
PORE SIZE MWCO (Da)	200,000	100,000	4,000
CONFIGURATION	tubular	tubular	Tubular
OPERATING PRESSURE (psi)			
typical	50-100	50-100	100 - 350
Maximum	150	150	440
MAX. OPERATING T(ºC)	80	80	80
ALLOWABLE pH	1.5 - 12.0	4.0 - 10.0	1.5 - 9.5

extracted 4 more times with the same procedure. The pooled extract was washed with 2 x 50 ml 10% sodium chloride and 3 x 50 ml water. The non-polar portion of this mixture (upper layer) was analyzed by HPLC. The HPLC separation was performed with a Waters system equipped with a Waters 717 plus auto-sampler, 2 Waters 515 HPLC pumps, a Waters 996 photodiode array detector PDA, and a Spectra Physics SP 8792 column heater. The data was stored and processed using a personal computer with the Waters Millennium 32 HPLC software. Each sample was analyzed in triplicate. Analyses were performed under dim light to prevent sample degradation by photo-oxidation. HPLC column used was a Carotenoid YMC polymeric C_{30} 4.6 mm i.d. x 250 mm 5-µm (YMC Inc., Wilmington, NC, USA). The mobile phase was methanol (solvent A) and methyl tert-butyl ether (solvent B) at a flow rate of 1.0 ml/min. The gradient procedure was as follows: 1) Initial conditions 62% solvent A and 38% solvent B kept 27 min

for column equilibration, 2) a 25 min linear gradient to 81.75% solvent B, 3) 2 min linear gradient to 100% solvent B, 4) 10 min with 100% solvent B for column cleaning. Column temperature was maintained at 30 °C. Standard solutions were prepared with all-trans-lycopene purchased from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were of HPLC grade. Before chemical analyses, the standard solutions and samples were filtered through Nalgene PTFE 0.22 μm filter.

4.3. Results and discussion

To study the membrane characteristics, the performance of the membranes is interpreted from the solvent flux and solutes rejection. The following calculations are used for the experiments:

Flux (J) is defined as:

$$J(LMH) = \frac{\text{Volume of permeate (ml) x 3600 (s) x 1 (L)}}{\text{1000 (ml) x Time (s) x 1 (h) x 0.0479 } (m^2)} \tag{4.1}$$

Rejection (R) is defined as:

$$R(\%) = \left(1 - \frac{C_P}{C_R}\right) \times 100 \tag{4.2}$$

where C_p is the concentration of the solute in the permeate and C_R is the concentration of the solute in the retentate.

The performance of the three membranes was evaluated by comparing rejection and flux under the same operating conditions. Screening was done using tomato juice containing 10.2 mg/100gr lycopene as the feed. Results are shown in Figure 4.3. Rejection was total (100%) for the three membranes. An explanation for this result is that lycopene was not released from the fiber matrix of the juice. The particle size of the vegetable fiber matrix was big enough to be rejected completely at 200,000 MWCO. Only soluble salts, sugars and small molecule proteins should have permeated through the membrane. Membrane FP 200 presented the best flux, 195 LMH at 50 psi TMP and 60 °C . FP 100 flux was 170 LMH at 50 psi TMP, ES 404 was 44 LMH at 100 psi. ES 404 was operated at higher pressure because at 50 psi TMP there was no flux due to the tighter pore of this nanofiltration membrane. The best performance was shown by FP 200 therefore, this membrane was selected for the batch concentrations.

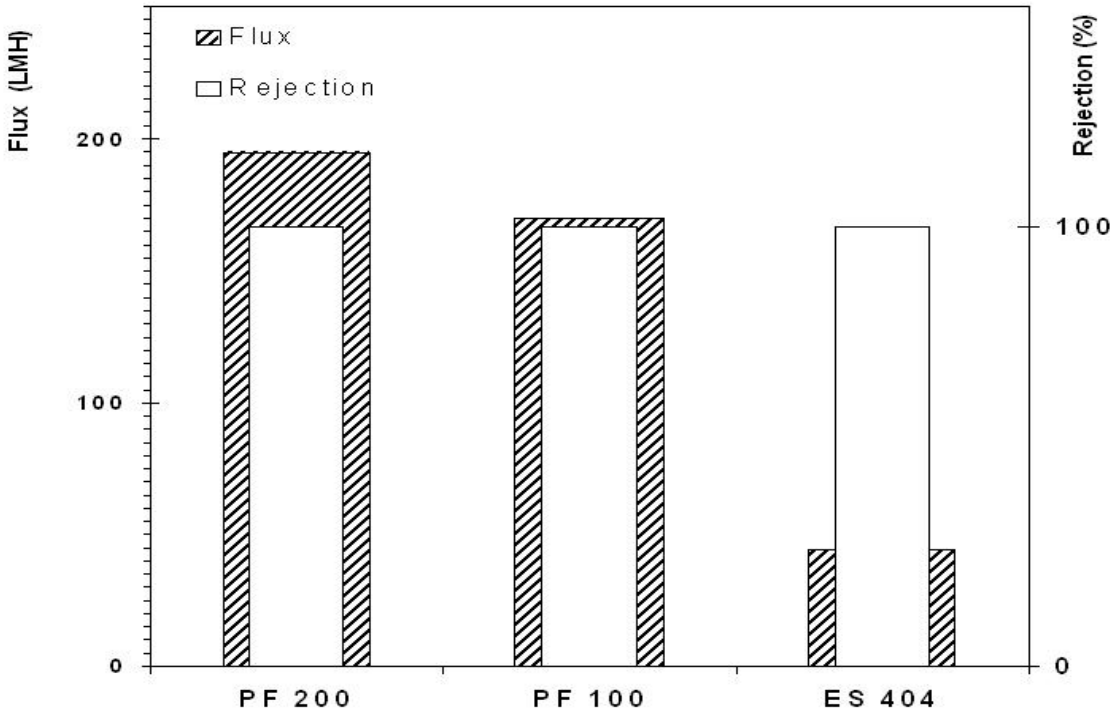

Figure 4.3 Flux and rejection for membranes tested. Temperature 60 ^{o}C, TMP 50 psi for FP100 and FP 200, 100 psi for ES 404. feed velocity 1.0 m/s.

Flux can be expressed in terms of a convective transport model:

$$J_v = \frac{L_p P}{\mu} \tag{4.3}$$

where J_v is the flux, L_p is the permeability coefficient of the membrane, P is the transmembrane pressure, and μ is the viscosity of the permeate. Membranes not affected by the solvent properties should give a linear plot of flux vs. 1/viscosity (Darcy's plot). A non-linear plot indicates the effect of solvent on membranes such as swelling of polymer and dilation of pores (Shukla 2000). For hydrophilic membranes, water is usually used for initial flux study and also for checking flux after cleaning.

The permeability can be calculated by simplifying equation 4.3 as follows:

$$J_v = AP \tag{4.4}$$

where J_v is solvent flux, A is solvent permeability at a particular temperature, and P is the applied transmembrane pressure. A is the slope in a plot of flux vs. TMP when the membrane material is not affected by the solvent and the flux is in the called pressure controlled region. Water permeability (A_w) for the membranes is reported in Figure 4.4. Water permeability at 20^{o}C was 30.92 LMH/psi for the FP 200, 19.1 LMH/psi for the FP 100, and 1.7 LMH/psi for the ES 404. Tomato juice fluxes are also presented as A values to shown the higher flow resistance due to the juice solutes. Water flux indicates the maximum flux achievable for the membrane.

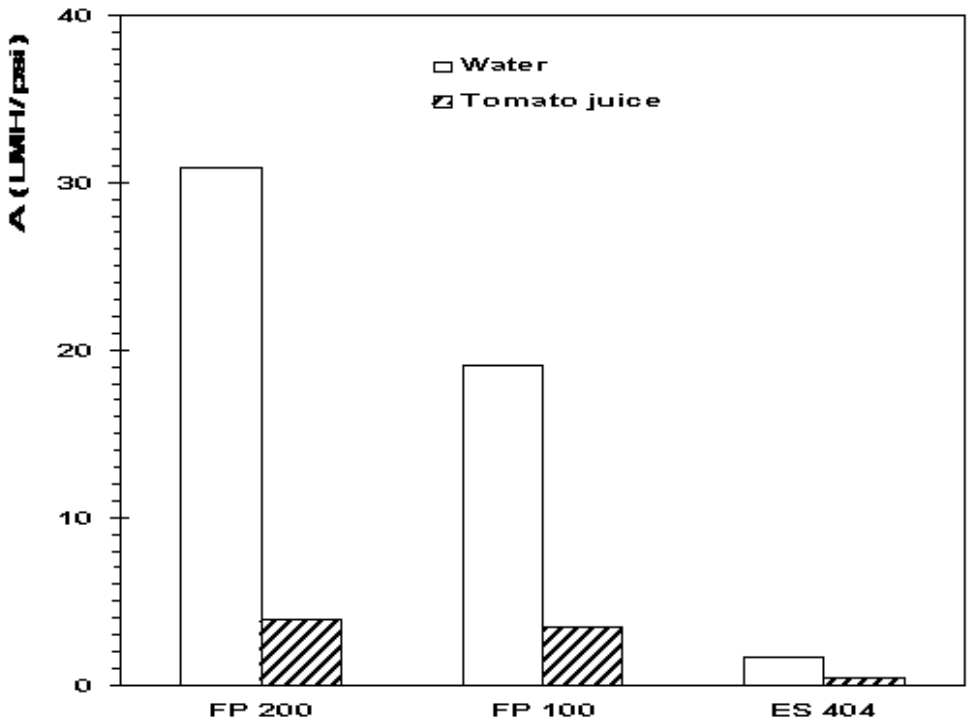

Figure 4.4 Permeabilities for membranes tested. Temperature 60 ^{o}C, feed velocity 1.0 m/s.

The performance of FP 200 membrane in concentrating lycopene from tomato juice at 50 psi TMP and 60 ^{o}C was evaluated up to VCR 4.5 which was the maximum reached by the unit. Figure 4.5 shows the typical lycopene concentration in retentate and permeate and the corresponding flux and rejection at 50 psi TMP. At VCR 4.5, the lycopene concentration went up from 11.2 mg/100g (the feed) to 51.7 mg/100g. This is an increase of up to 462% of the

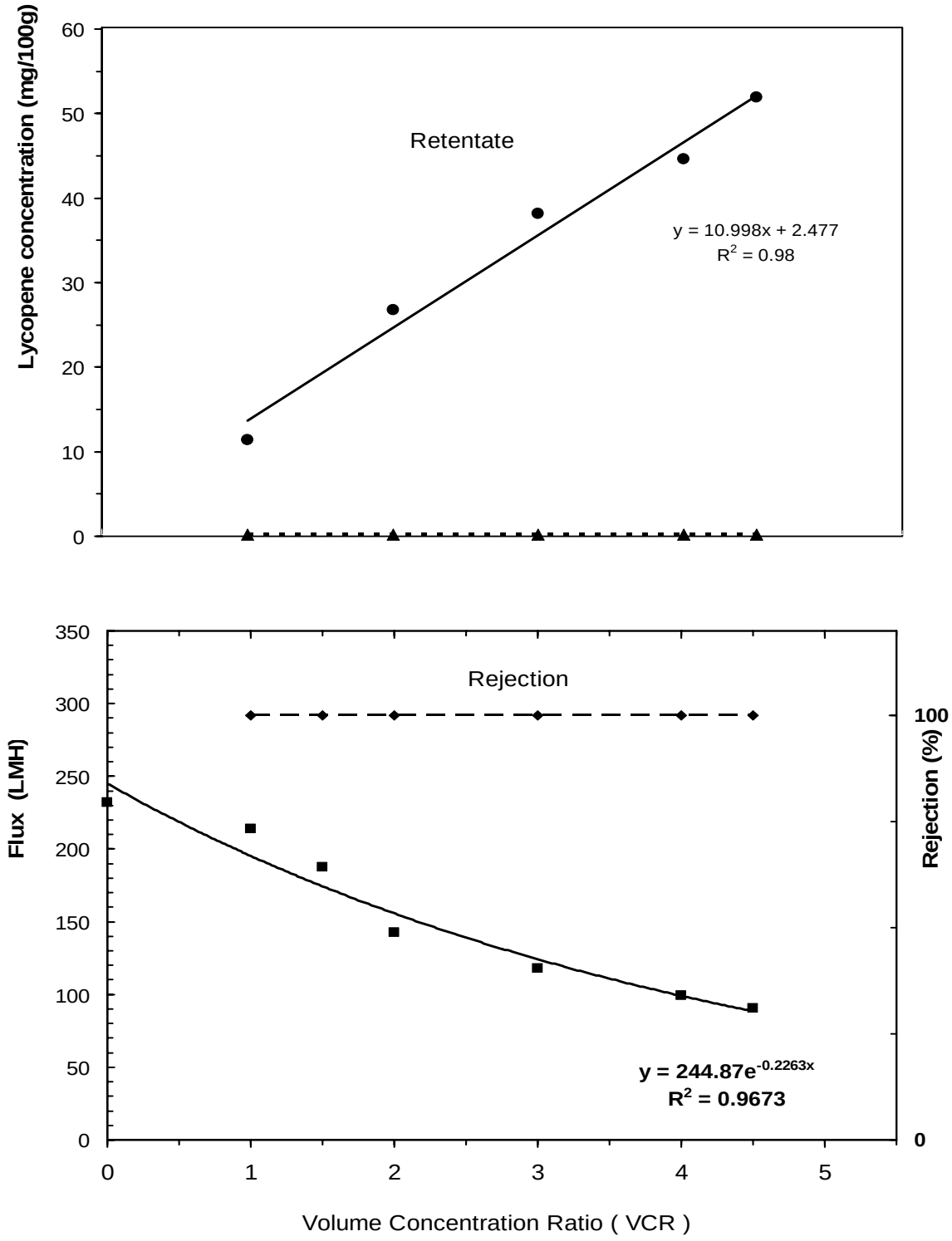

Figure 4.5 Performance of FP 200 membrane with commercial tomato juice. Temperature 60°C, TMP 50 psi, velocity 1 m/s

concentration in the feed. In Figure 4.6 is shown a mass balance of the process carried out. UF removed 80% of the water contained in the tomato juice being fed.

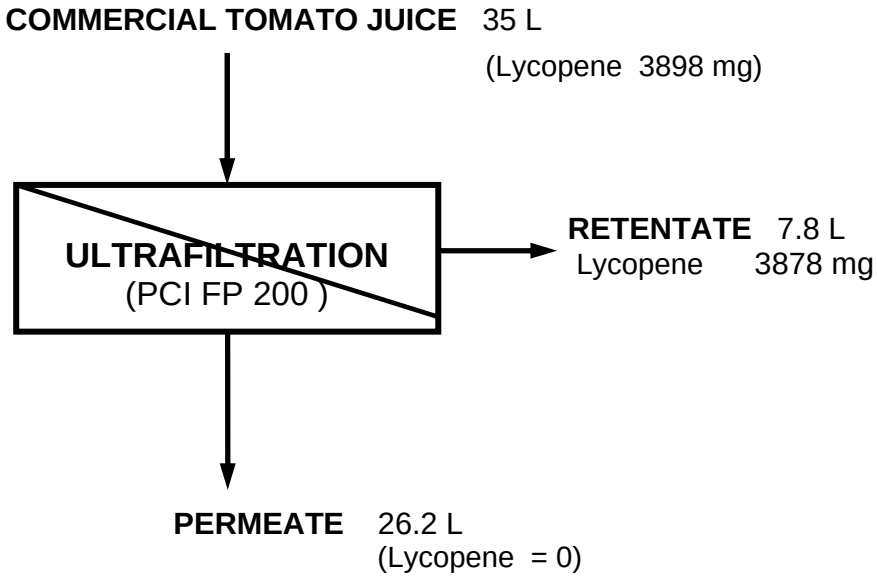

Figure 4.6 Mass balance for the ultrafiltration concentration. Membrane PCI FP 200, temperature 60 ^{o}C, TMP 50 psi, velocity 1.0 m/s

4.4. Conclusions

The results shown in this study indicate that UF concentration of lycopene from tomato juice is technically feasible. Three polymeric membranes (PCI FP 200, PCI FP 100, and PCI ES 404) were screened with tomato juice containing 10 to 11 mg of lycopene for 100g of sample. Lycopene was not detected in the permeate stream with the analysis performed, indicating a total rejection of lycopene for the three membranes tested. PCI's FP 200 membrane was the best in terms of flux. The FP 200 membrane resulted in an average flux at 60°C and 50 psi TMP of 155 LMH when concentrating lycopene from 11.2 mg/100g to VCR 4.5. A ultrafiltration stage can remove up to 80% of the water content from the feed. The tomato paste obtained as retentate contains 51.7 mg lycopene/100g sample.

5. SOLVENT EXTRACTION OF LYCOPENE FROM TOMATO PASTE

5.1. Introduction

The preparation of crystalline lycopene of high purity from fruits and vegetables generally requires an initial extraction with organic solvents, and then various steps of purification. Acetone (AC), ethanol (EtOH), ethyl acetate (EA), hexane (Hex), isopropanol (IPA), methanol, and propanol are suitable solvents mentioned in several patents for lycopene extraction. Among them, ethanol, ethyl acetate and hexane have been found as particularly satisfactory for lycopene extraction processes.

The objective of this study was to determine the solvent with best extraction efficiency from tomato paste and membrane material compatibility, to be used in the NF separation. The solvents chosen for the study were EtOH, EA, Hex, IPA, and tetrahydrofuran (THF). Isopropanol is an alcohol to which several commercial membranes are stable. Tetrahydrofuran is widely used in laboratory extractions due to its higher recoveries.

5.2. Materials and methods

Tomato paste was obtained by UF concentration from commercial tomato juice. All-trans lycopene and β-carotene standards were obtained from Sigma Co. Chemical, St. Louis, MO, USA. HPLC and reagent grade solvents AC, EtOH, EA, Hex, IPA, and THF were obtained from Fisher's scientific. Properties of the solvents used are shown in Table 5.1.

Extractions at lab scale were performed based in the procedure of Taungbodhitham and others (1998). The extraction procedure is summarized in Figure 5.1. Briefly, 5 g tomato paste sample was extracted in a plastic Falcon tube with an Ultra-Turrax T25 homogenizer at 16,000 rpm for 3 minutes using 88 ml cosolvent:hexane (4:3). Extract was filtered under reduced pressure in a Büchner funnel and collected in a separation funnel. Residue was re-extracted 4 more times following the same procedure using 88 ml solvent for the second wash and 50 ml for each of the subsequent washes. The pooled extract was washed 2 times with 100 ml 10% sodium chloride and 3 times with 100 ml water. The non-polar portion of this mixture (upper layer) was analyzed by HPLC. The HPLC separation was performed with a Waters system equipped with a Waters 717 plus auto-sampler, 2 Waters 515 HPLC pumps, a Waters 996 photodiode array detector PDA, and a Spectra Physics SP 8792 column heater. The data was stored and processed

Table 5.1- Physical properties for the solvents used in the experiments. (Daubert and Danner 1989; Snyder 1974).

Solvent	Acetone (CH_3COCH_3)	Ethanol (C_2H_5OH)	Isopropanol ($C_3HCHOHCH_3$)	Ethyl Acetate ($COOC_2H_5$)	THF (C_4H_8O)	Hexane (C_6H_{14})
Molecular weight	58.08	46.07	60.09	88.1	72.1	86.18
Density (g/ml)	0.7899	0.7894	0.7851	0.9020	1.4074	0.6594
Viscosity (cPoise)	0.32	1.2	2.3	0.45	0.55	0.33
Polarity index	5.1	4.3	3.9	4.4	4.0	0.0
Boiling point (oC)	56.2	78.3	82.5	77.0	66.0	68.7
Water solubility (%w/w)	100	100	100	8.7	100	0.001
Dielectrical constant	20.7	24.3	18.3	6.0	n.a.	1.9

n.a. = not available

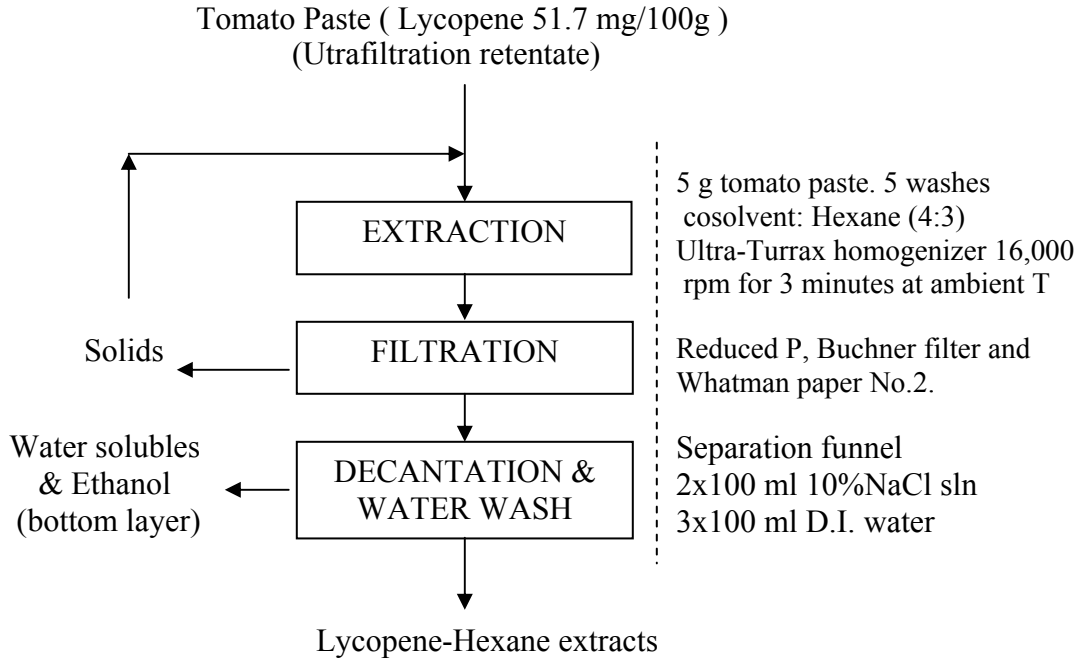

Figure 5.1 Schematic diagram for lycopene concentration and extraction.

using a personal computer with the Waters Millennium 32 HPLC software. Each sample was analyzed in triplicate. Analyses were performed under dim light to prevent sample degradation by photo-oxidation. HPLC column used was a Carotenoid YMC polymeric C_{30} 4.6 mm i.d. x 250 mm 5-µm (YMC Inc., Wilmington, NC, USA). The mobile phase was methanol (MeOH, solvent A) and methyl tert-butyl ether (MTBE, solvent B) at a flow rate of 1.0 ml/min. The gradient procedure was as follows: 1) Initial conditions, 62% solvent A and 38% solvent B, kept 27 min for column equilibration, 2) a 25 min linear gradient to 81.75% solvent B, 3) 2 min linear gradient to 100% solvent B, 4) 10 min with 100% solvent B for column cleaning. Column temperature was maintained at 30 °C. Carotenoids were monitored at λ 450 nm. Standard solutions and samples were filtered through Nalgene PTFE 0.22 µm filter. If necessary, samples were stored at -80°C before analysis. Figures 5.2 and 5.3 show the calibration curve of standard all-trans lycopene and all-trans-β-carotene.

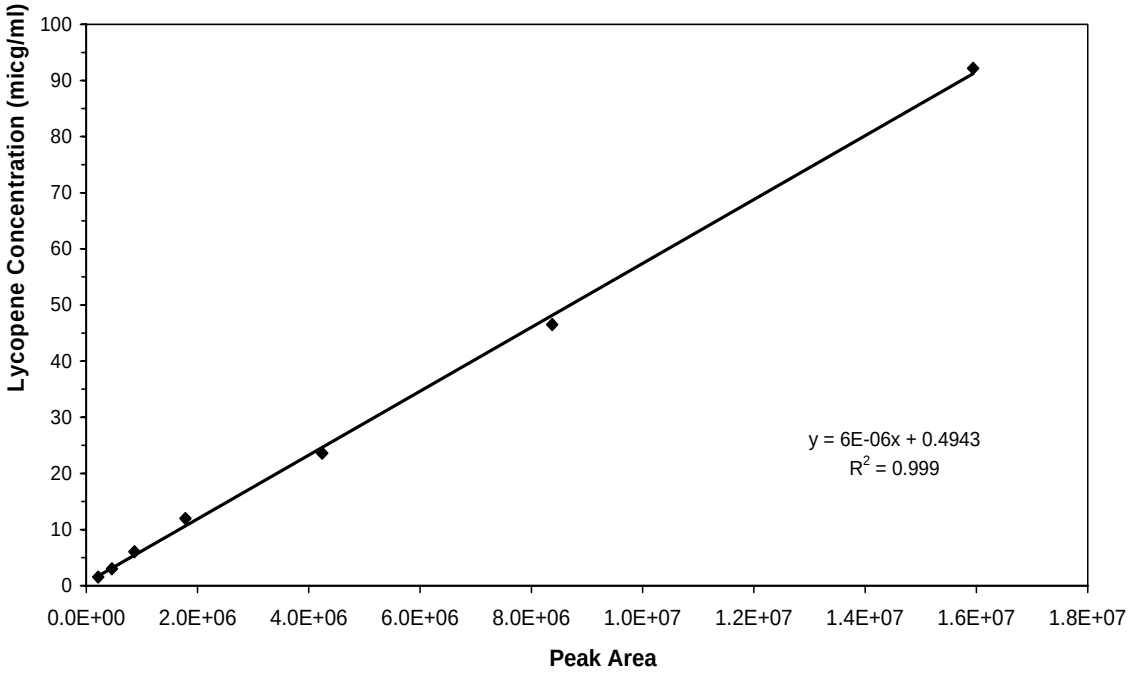

Figure 5.2 Calibration curve of absorbance by HPLC vs. standard lycopene solution.

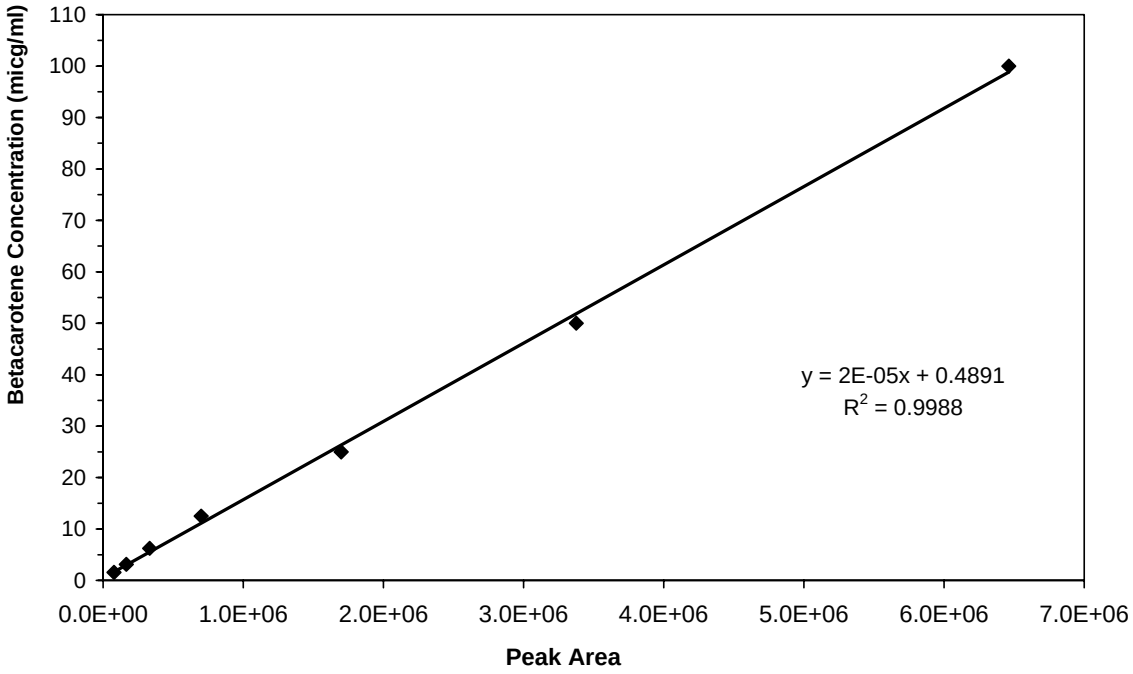

Figure 5.3 Calibration curve of absorbance by HPLC vs. standard β-carotene solution.

The identification of all-trans-lycopene and all-trans-β-carotene was carried out by comparing the retention times and absorption spectra with reference standards. In addition, the cis isomers were identified by comparison with the absorption spectrum characteristics shown in table 5.2. The resulting response factors of all-trans-lycopene or all-trans-β-carotene were used to calculate the contents of all the according geometrical isomers. Therefore, isomers with a lower specific absorption than the all-trans-isomer were somewhat underestimated (Hengartner and others 1992).

Table 5.2- Spectral characteristics of β–carotene and selected lycopene isomers found in tomato products. (Schierle and others 1997; Breitenbach and others 2001).

Carotenoid assignment	Darray data of HPLC peaks (nm)				Relative absorption of cis peak[b]
	Cis peak	Absorption maxima[a]			
β-carotene[c]	338	428	**452**	491	0.05
5,13'-cis-lycopene[c]	362	445	**460**	490	n.a.
13-cis-lycopene[d]	360	437	**463**	494	0.55
9-cis-lycopene[c]	362	442	**467**	497	0.15
7-cis-lycopene[c]	363	445	**469**	501	0.09
5,5'-cis-lycopene[d]	363	444	**470**	502	0.06
all-trans-lycopene[c]	363	446	**472**	504	0.07
5-cis-lycopene[d]	363	446	**473**	504	0.07

[a] Main maxima are in bold face.

[b] Relative absorption of cis-peak means absorption at the subsidiary peak (at aprox. 360 nm) divided by the absorption at main maxima (in bold face).

[c] From Breitenbach and others 2001.

[d] From Schierle and others 1997.

5.3. Results and discussion

Tomato paste HPLC chromatogram is shown in Figure 5.4. β-carotene, 13-cis-lycopene, 7-cis-lycopene, and all-trans-lycopene, were identified. Figures 5.5, 5.6, 5.7 and 5.8 show obtained PDA absorption for the compounds identified. Retention time for all-trans-lycopene was 27.032 min., for 7-cis-lycopene was 22.606 min., for 13-cis-lycopene was 17.912 min., and for β-carotene was 8.539 min.

Figure 5.9 shows the all-trans-lycopene recovery obtained with each of the tested solvent mixtures. Ethanol:hexane presented the highest recovery (51.7 mg/100g tomato paste). Recoveries obtained with EtOH:Hex and THF:Hex was not significantly different ($p<0.01$).

Figure 5.10 shows percentage of all-trans-lycopene recovery for every of the 5 washes used in the extraction and for each of the tested solvent mixtures. Percentages were calculated relative to 51.7 mg/100g sample, the highest recovery obtained with EtOH:Hex. Ethyl acetate:hexane and THF:Hex presented the higher efficiency with the first wash (equivalent to 17.5 ml/g solvent to solids ratio). This result can be attributed to the solubility between cosolvent and hexane. At the third wash, equivalent to 45 ml/g solvent to solids ratio, EtOH and EA presented the same efficiency, with a lycopene recovery of 90%. In the fourth and fifth washes (55 and 65 ml/g solvent to solids ratio) EtOH and THF presented better efficiency. This better result is probably because at lower lycopene concentrations, disruption of the hydrophilic vegetable matrix becomes more important in releasing and dissolving the bioactive compound.

Figure 5.11 shows hexane extracts of the five washes in a typical EtOH:Hex extraction. Average extract concentrations were 44.3, 29.7, 19.7, 10.0 and 4.9 μg/ml for the 1st, 2nd, 3rd, 4th, and 5th wash respectively.

Figure 5.12 shows the β-carotene recovery obtained with each of the tested solvent mixtures. Ethanol:hexane presented the highest recovery (2.9 mg/100g tomato paste).

Amounts of 13- cis-lycopene and 7-cis-lycopene were estimated by using HPLC peak response of all-trans-lycopene standard. 13-cis-lycopene was 1.9 mg/100g tomato paste and 7-cis-lycopene was 1.3 mg/100g tomato paste. The rest of unidentified lycopene cis-isomers were 1.0 mg/100 tomato paste. Total carotenoids were 58.8 mg/100g tomato paste where the all-trans-lycopene counts for 87.9% (51.7 mg/100g tomato paste).

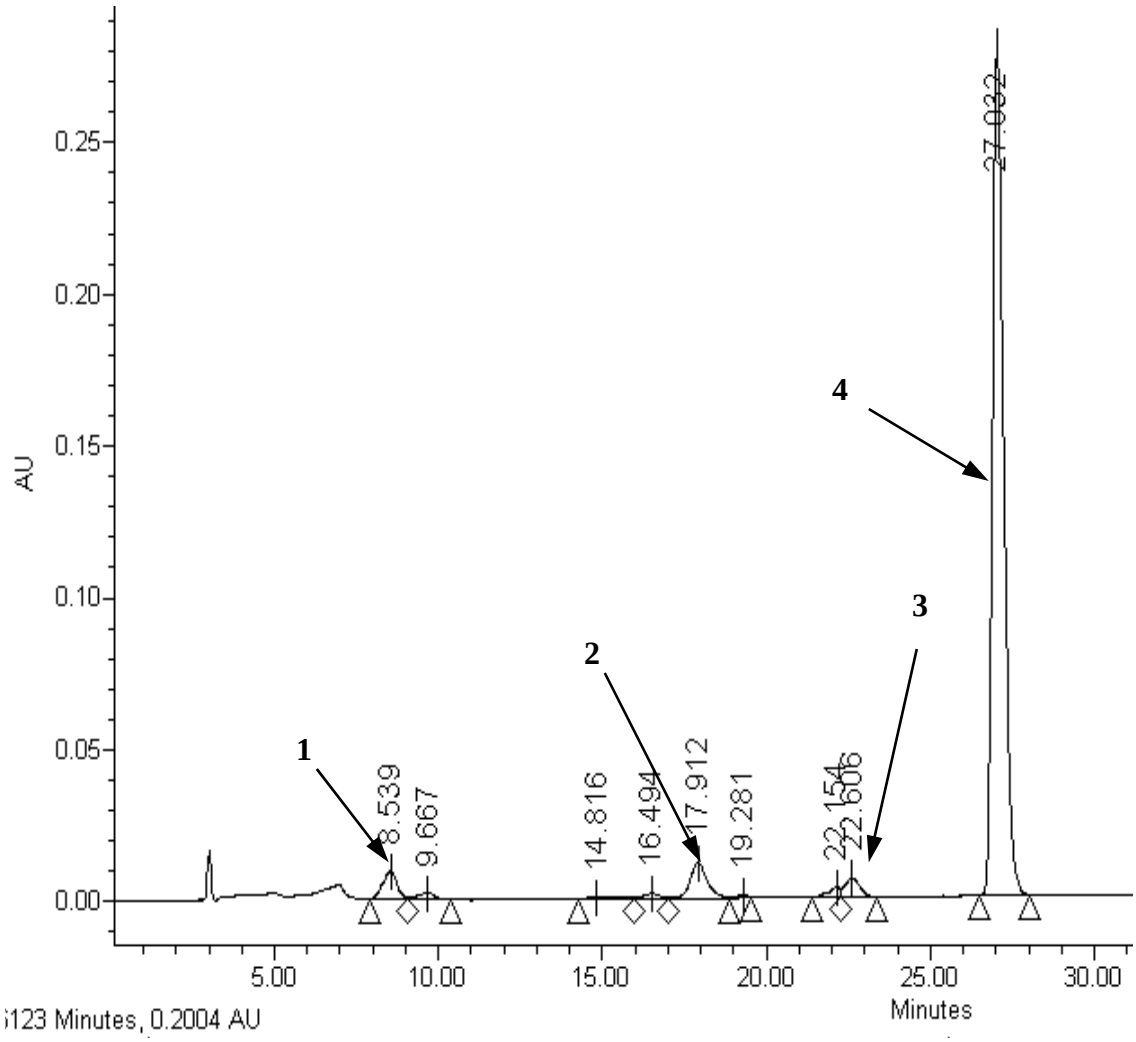

Figure 5.4 HPLC chromatogram of tomato paste. Peaks: 1=β-carotene, 2=13-cis-lycopene, 3= 7-cis-lycopene, 4= all-trans-lycopene.

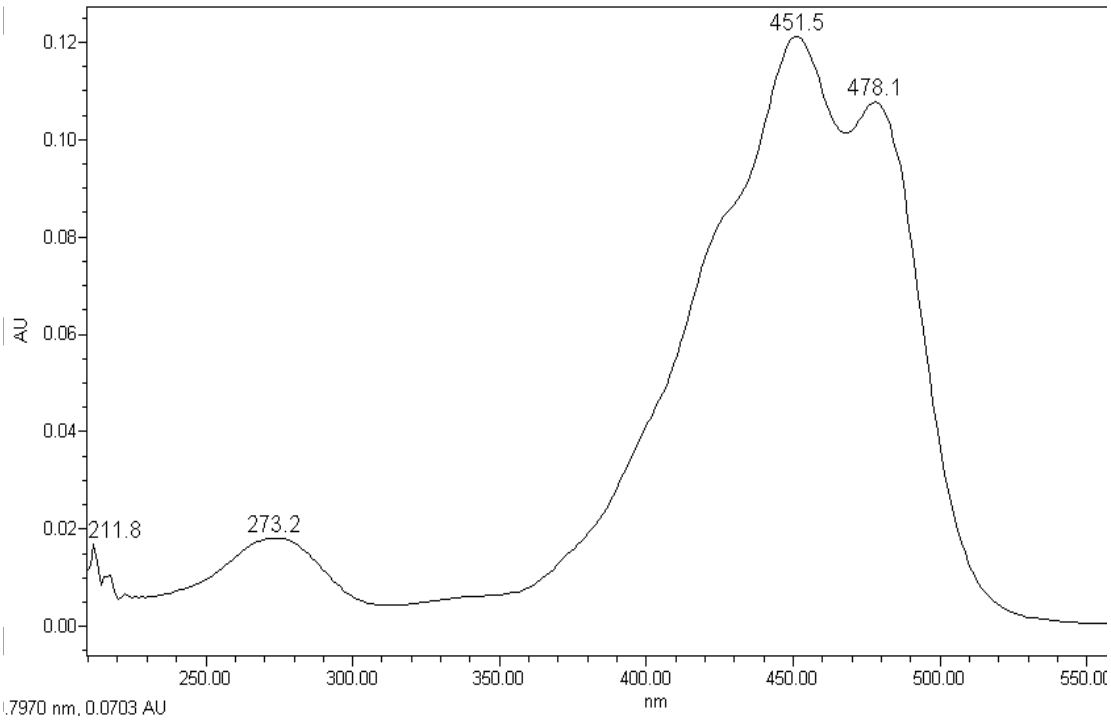

Figure 5.5 Absorption maxima of β-carotene

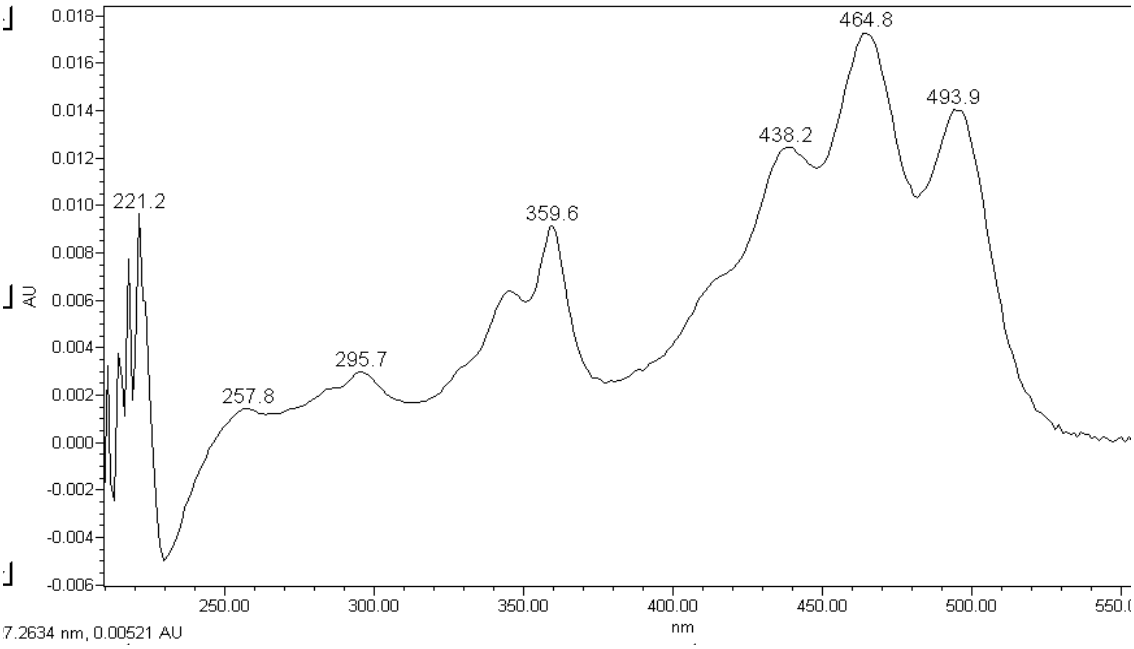

Figure 5.6 Absorption maxima of 13-cis-lycopene.

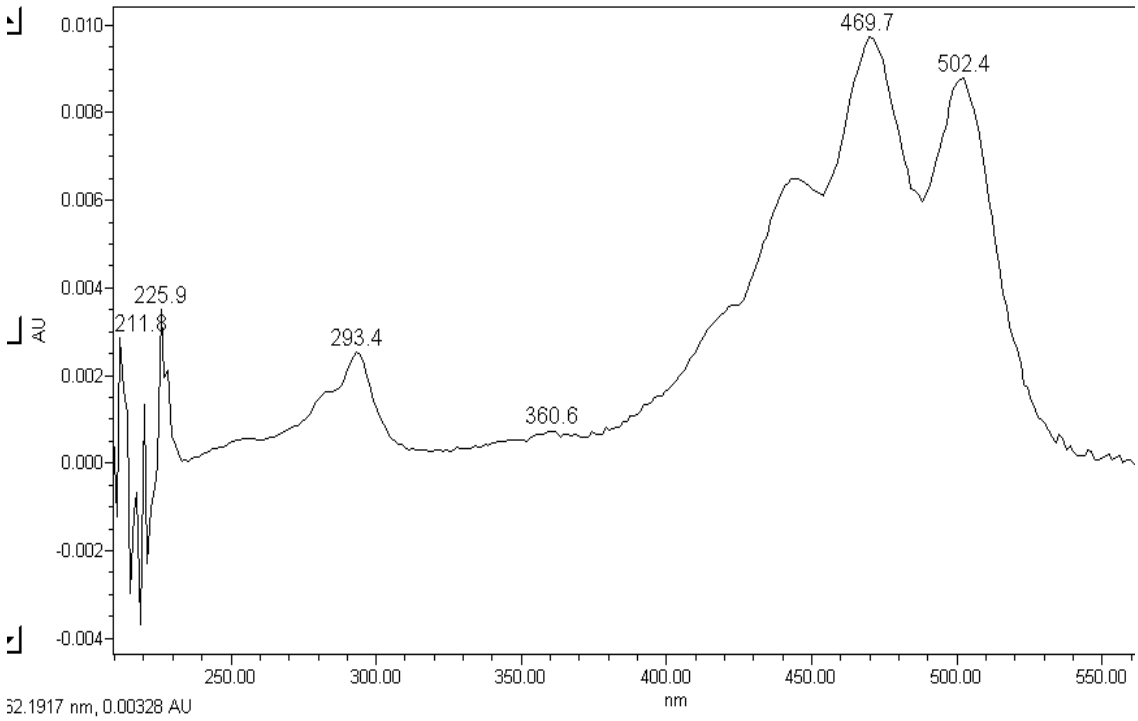

Figure 5.7 Absorption maxima of 7-cis-lycopene.

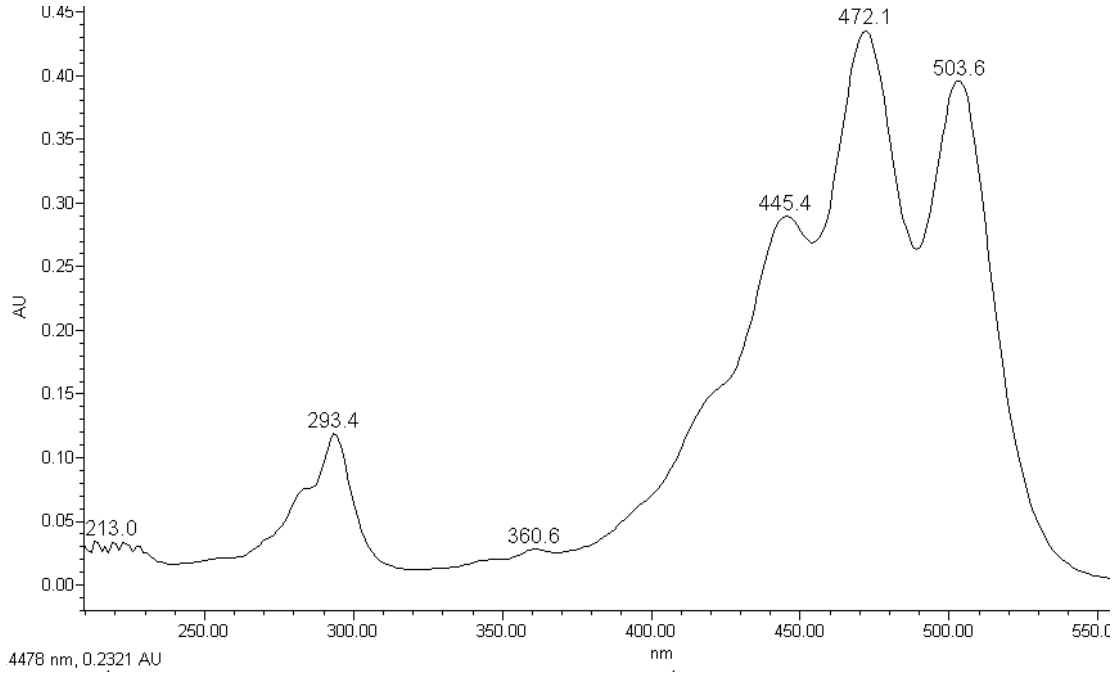

Figure 5.8 Absorption maxima of all-trans-lycopene.

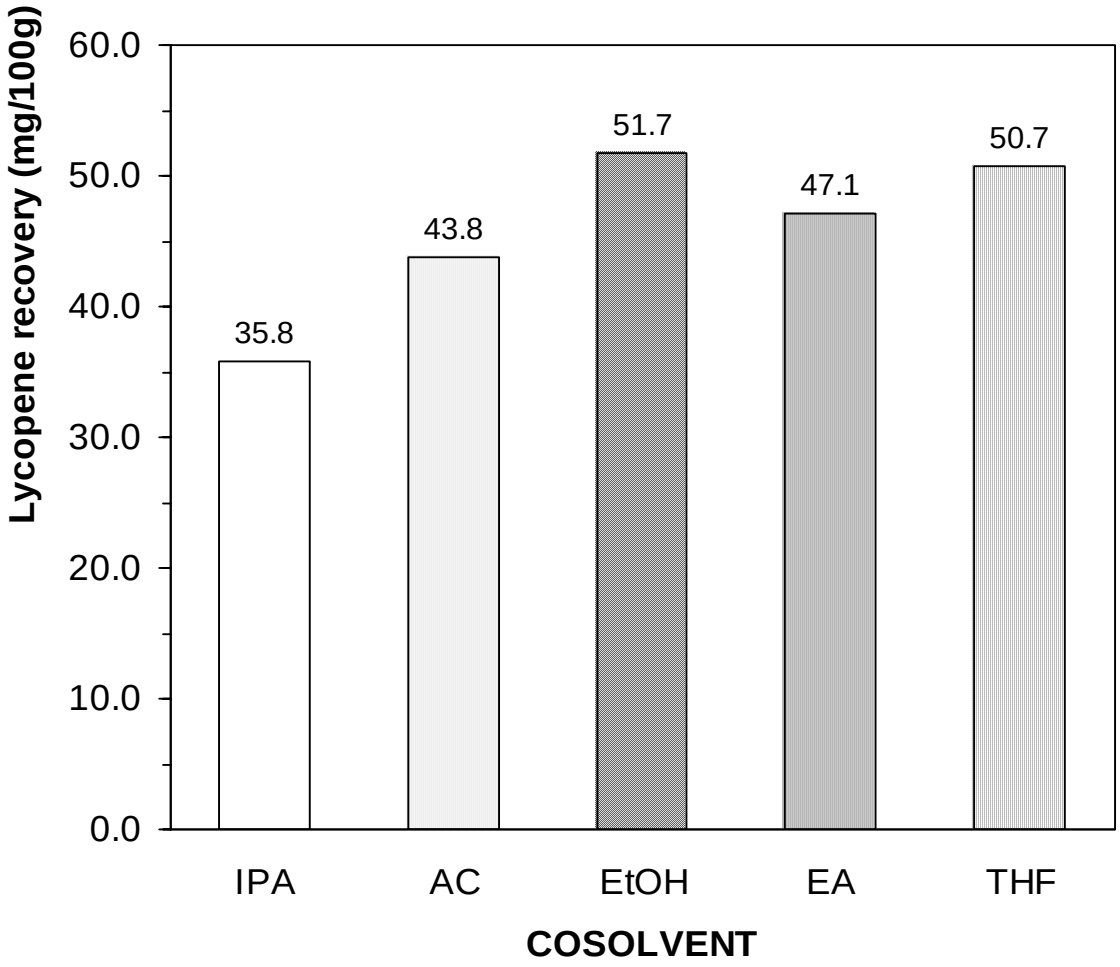

Figure 5.9 Lycopene recovery for each cosolvent used. IPA=isopropanol, AC= acetone, EtOH= ethanol, EA= ethyl acetate, THF= tetrahydrofuran. Cosolvent:Hexane (4:3 v/v). 65 ml solvent mix/g sample.

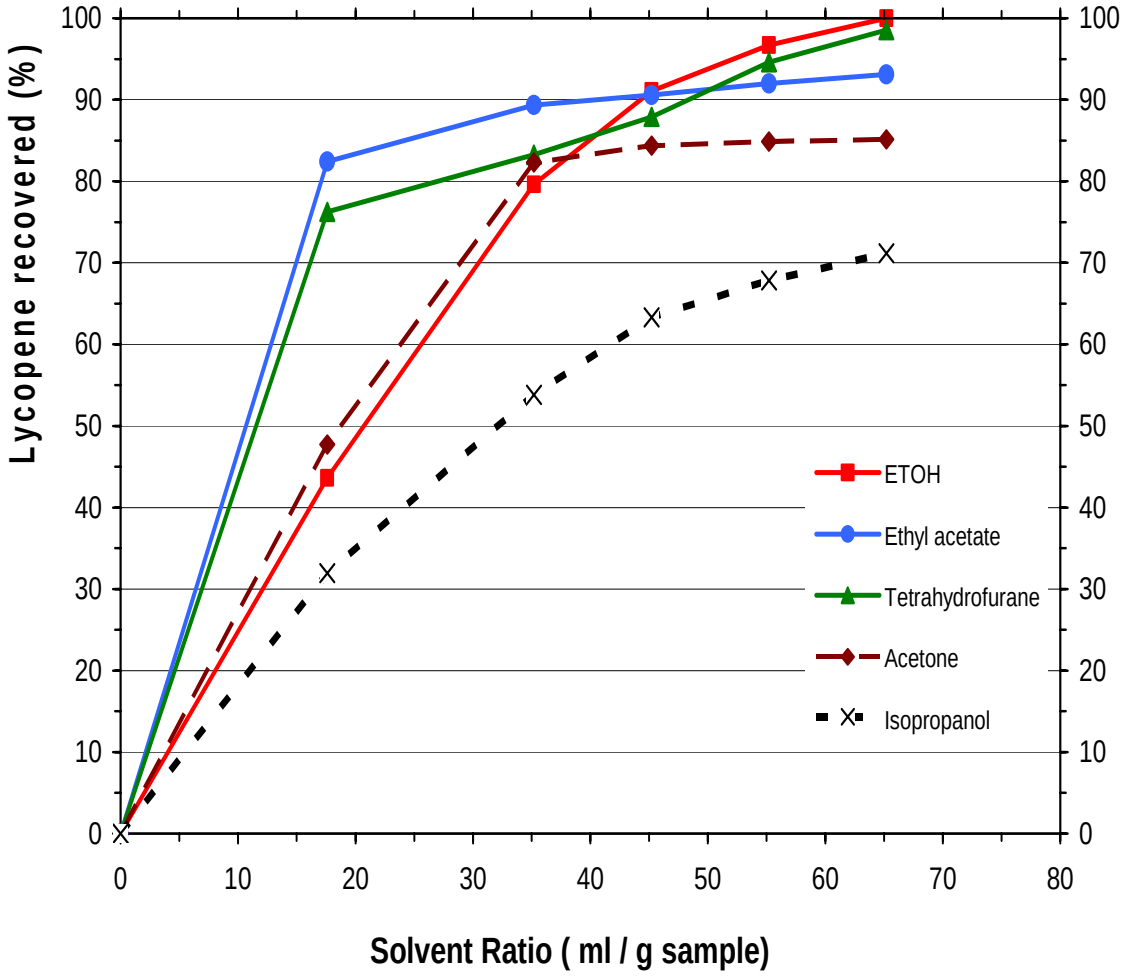

Figure 5.10 Percentage of recovery for each of the 5 extraction washes expressed as solvent to solids ratio (ml/g sample). EtOH:hexane 100%.

Figure 5.11 Typical lycopene-hexane extracts for 1st, 2nd, 3rd, 4th, and 5th wash. Solvent mix EtOH:Hex (4:3).

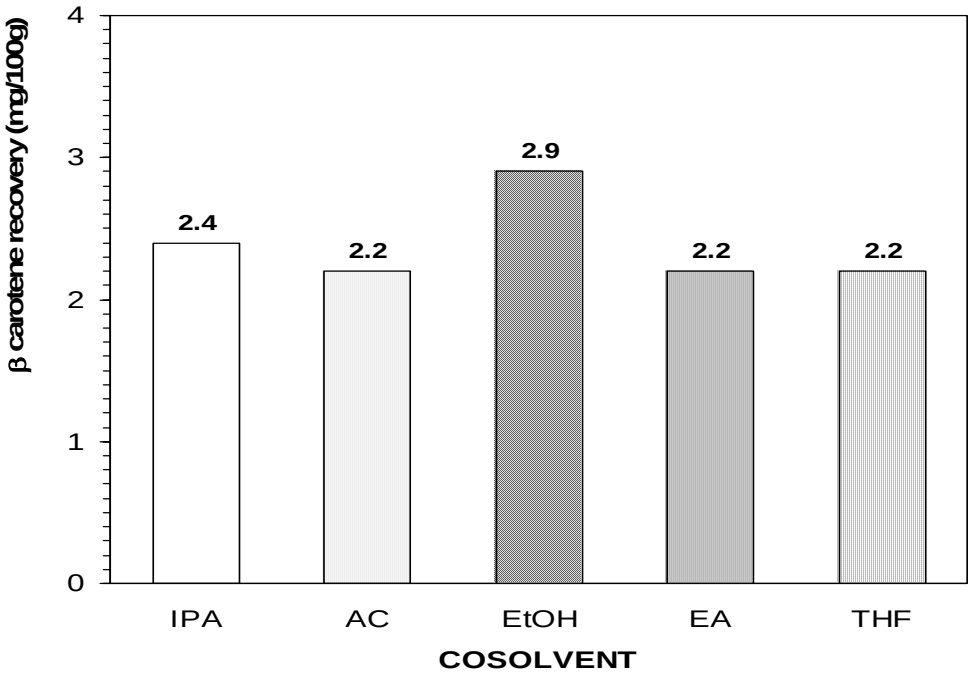

Figure 5.12 β-carotene recovery for each cosolvent used. IPA=isopropanol, AC= acetone, EtOH= ethanol, EA= ethyl acetate, THF= tetrahydrofuran. Cosolvent:Hex (4:3 v/v). 65 ml solvent mix/g sample.

5.4. Conclusions

Five solvents (ethanol, ethyl acetate, tetrahydrofuran, acetone, and isopropanol) were tested at ambient temperature (21-23 °C) as cosolvents with hexane for extraction of lycopene from tomato paste obtained by UF. Ethanol:hexane (4:3) presented the highest recovery (51.7 mg of all-trans-lycopene / 100g of sample). Multiple extractions of the same tomato paste with fresh ethanol:hexane resulted in an all-trans-lycopene yield of 91% after 3 extractions at a solvent-to-solids ratio of 45 ml per g. Total recovery of carotenoids in the tomato paste was estimated in 58.8 mg/100g where all-trans-lycopene counted for 87.9%, β-carotene for 4.9%, 13-cis-lycopene for 3.2%, 7-cis-lycopene for 2.2%, and unidentified lycopene cis-isomers for 1.7%. Regarding the membrane compatibility to the best solvent mix ethanol:hexane, several commercial membranes are been offered as resistant to EtOH (Osmonics DS 5, Filmtec NF 45, Koch MPF 50,60 and 44). Also, there are recent studies on membrane stability and performance using hexane as the permeating solvent (Van der Bruggen and others 2002; Machado and others 1999; Raman 1996a, 1996b; Koseoglu and others 1990).

6. MEMBRANE SCREENING FOR RECOVERY OF LYCOPENE FROM HEXANE EXTRACTS

6.1. Introduction

The use of polymeric NF membranes for separations in organic solvents has been suggested by a growing number of authors (Machado and others 1999; Schmidt and others 1999; Whu and others 1999; Uragami and others 1978), but practical application is usually not yet possible because of limited solvent stability (Van der Bruggen and Vandecasteele 2002), lower solute rejection in comparison with the rejections obtained in aqueous solution (Nwuha 2000) and low solvent fluxes at enhanced solute concentrations (Whu and others 2000). Important separation problems such as the concentration of amino acids in organic solvents (Reddy and others 1996) could therefore not yet be solved satisfactorily. Moreover, application of NF in hybrid processes with pervaporation and crystallization for use in the pharmaceutical industry, as suggested by Hestekin and others (1999), depends on the availability of solvent-stable NF membranes. The same is true for other combined processes, e.g. the use of NF as a preconcentration step before evaporation in order to recover valuable pharmaceutical products from solvent streams. Current research focuses on obtaining improved solvent stability by the development of new polymeric membrane materials (Musale and Kumar 2000; Golemme and Drioli 1996), or by the use of ceramic NF membranes (Guizard and others 2000; Blanc and others 1998; Benfer and others 2001). Additionally, the mechanism of separations with polymeric membranes in organic solvents is poorly understood. Performance of NF membranes in organic solvents is very different from that in aqueous solution; fluxes and rejections differ significantly. Prediction of the behavior of a membrane in an organic solvent remains difficult.

There have been no reports on the use of membrane technology for recovering valuable minor components such as lycopene from tomatoes or fruits and vegetables. The objective of this research was to recover lycopene from lycopene-solvent solutions using nanofiltration membrane separation. In this study, qualitative and quantitative comparison between hexane and lycopene-hexane solutions fluxes and rejections for five membranes (Osmonics DS-7 and DS-5, Koch MPF50, PCI ES-404, and Filmtec NF-45) was analyzed. Rejection and flux as influenced by pressure, temperature and solute concentration was studied for the best membrane Osmonics DS-7.

6.2. Materials and methods

Separation experiments were carried out with a solvent compatible NF bench-scale unit (Fig. 6.1). The unit could be used up to 1000 psig and temperatures up to 177°C. A cross-flow Osmonics SEPA-CF membrane cell which could accommodate flat-sheet rectangular coupons of 19 cm x 14 cm was used. The effective surface area was 0.0126 m^2. The membrane module is shown in Figure 6.2. For tubular membranes a PCI single tube cell was used replacing the Osmonics SEPA-CF flat sheet cell. Transmembrane pressures (TMP) were 100, 200, 300, 400, 500, and 600 psig. The flow and TMP are set manually by means of a frequency variator and valves installed at the inlet and outlet of the membrane cell. The retentate is recycled to the feed tank and the permeate can be recycled as well. Samples can be taken at any time in the experiment. The temperatures were 26.7, 34.4, and 40°C. A heat exchanger with supply of water and steam was used to keep the temperature stable. Flux and rejection values are an average of at least three measurements with the same membrane sheet/tube; flux measurements were carried out three times for different membranes of the same type.

The membranes used were DS5-DK and DS-7, obtained from GE Osmonics (Minnetonka, MN, USA), MPF 50 obtained from Koch Membrane Systems (Wilmington, MA, USA), PCI ES-404 from PCI Membrane Systems Inc. (Milford, OH, USA), and NF-45 from Filmtec Corporation (Minneapolis, MN, USA). The information provided by the manufacturers is summarized in Table 6.1. All membranes, except PCI ES-404, were rated as NF membranes. The pretreatment of membranes supplied “semi-dry” or wet with water (DS5-DK, DS-7, NF-45, and PCI ES-404) was as follow: The coupon/tube was thoroughly washed to remove the preservatives and humectants by holding it under running deionized (DI) water. The individual coupon was then pressurized in the test cell and several ml of DI water allowed permeating through the membrane to wash off any preservatives in the pores. This cleaning and pressure testing was usually done a few times until the water flux was reproducible. The coupon was then soaked in 50% (v/v) ethanol in water for at least 3 hours at room temperature. The membrane was then placed in the cell and 50% (v/v) ethanol in water was allowed to permeate for at least 30 minutes. The membrane was then conditioned with 100% ethanol, 50% ethanol in hexane, and 100% hexane allowing each solvent to permeate for at least 30 minutes. Koch membrane MPF-50 was shipped in 50% aqueous ethanol. Cleaning of this membrane was done with 50% ethanol in water and then conditioning with 100% ethanol, 50% (v/v) ethanol/hexane, and 100% hexane for at least 30 minutes for each solvent.

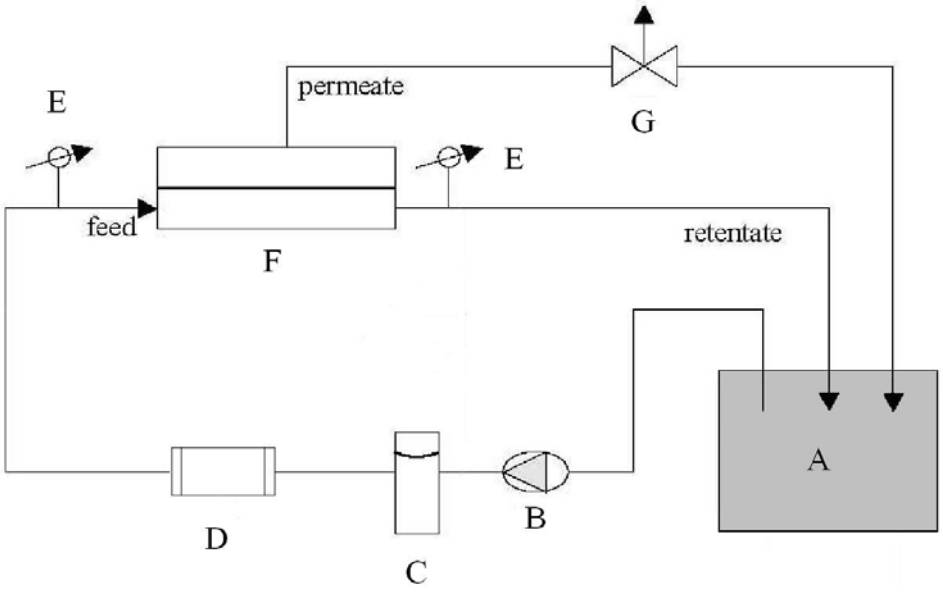

Figure 6.1 Schematic of bench-scale nanofiltration equipment. (A = feed tank; B= pump with frequency variator; C= pulsation damper; D = heat exchanger; E= gauges; F = membrane cell; G = permeate sampling valve).

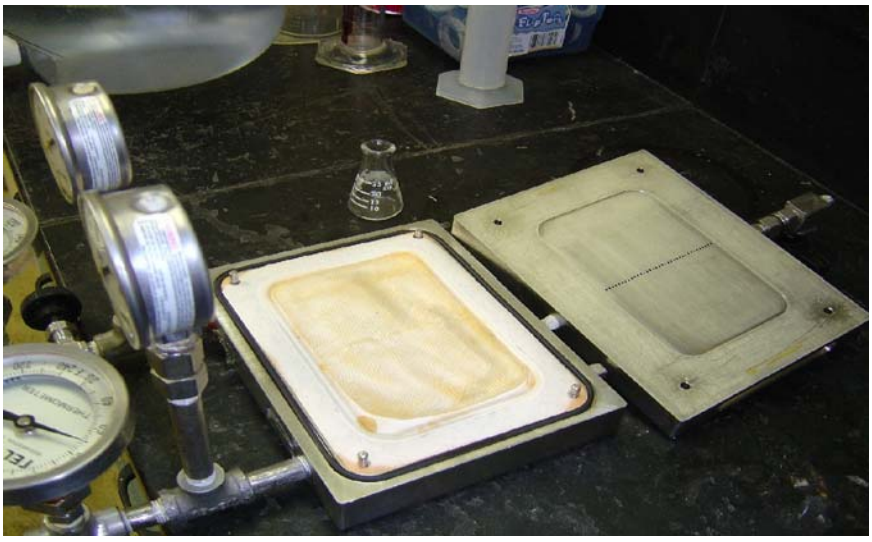

Figure 6.2 Membrane module Osmonics Sepa CF used in experiment.

Table 6.1- Nanofiltration membrane characteristics as indicated by the manufacturers.

MEMBRANE	OSMONICS DESAL DS-7	KOCH MPF-50	PCI ES-404	FILMTEC NF 45	OSMONICS DS-5- DK
TYPE	Nanofiltration Hydrophobic	Nanofiltration Hydrophobic	Ultrafiltration Hydrophobic	Nanofiltration Hydrophilic	Nanofiltration Hydrophilic
MATERIAL	Thin film polymer	Thin film polymer	Polyethersulphone	Polypiperazine Amide Thin Film	Thin film polymer
SOLVENT RESISTANCE					
Alcohols	Good	Stable	Limited Stability	Not Stable	Stable
Hexane	Good	Stable	Limited Stability	Not Stable	Not reported
PORE SIZE MWCO (Da)	Not rated	700	4000	200	300
CONFIGURATION	Flat sheet	Flat sheet	Tubular	Flat sheet	Flat sheet
OPERATING PRESSURE (psi)					
typical	-	220 - 515	100 - 350	220 - 515	70 - 400
Maximum	800	590	440	1200	500
MAX. OPERATING T(ºC)	90	40	60	45	70
ALLOWABLE pH					
Continuous operation	1.0 - 12.0	4.0 - 10.0	1.5 - 9.5	3.0 - 10.0	2.0 - 11.0
Clean-In-Place	-	2.0 - 11.0	-	1.0 - 11.5	1.0 - 11.5

Physical properties for water, ethanol and n-hexane are summarized in Table 6.2. (Daubet and Danner 1989; Snyder 1974). Solvents were purchased from AAPER Alcohol and Chemical Company (Shelbyville, KY, USA) and Tem-Tex Solvents Co. (Temple, TX, USA).

Lycopene-hexane solutions were prepared starting with name-brand and store-brand canned tomato juice purchased in local stores. The lycopene concentration and solvent extraction process is summarized in Figure 6.3. Tomato juice was first filtered in a pilot plant UF unit as described in detail in section 4. Then, 200 to 300 g of tomato concentrate from the UF process, was then extracted with 2.4 L ethanol:hexane (4:3). The mixture was stirred for 30 minutes at ambient temperature (20-23°C). After extraction, the solids were removed from the ethanol:hexane by filtering under reduce pressure through a Whatman #2 paper in a Buchner filter. Solid residue was re-extracted 2 more times following the same procedure. Total ratio solvent mixture to tomato paste was 35:1 (v:w). The pooled extract was washed with 2 x 500 ml 10% sodium chloride and 2 x 500 ml DI water in a separation funnel and the non-polar portion (upper layer) was collected. This lycopene-hexane extract was used as the feed for the NF membrane unit.

Lycopene content was determined by high performance liquid chromatography (HPLC). The HPLC separation was performed with a Waters system equipped with a Waters 717 plus auto-sampler, 2 Waters 515 HPLC pumps, a Waters 996 photodiode array detector PDA, and a Spectra Physics SP 8792 column heater. The data was stored and processed using a personal computer with the Waters Millennium 32 HPLC software. Each sample was analyzed in triplicate. Analyses were performed under dim light to prevent sample degradation by photo-oxidation. HPLC column used was a Carotenoid YMC polymeric C_{30} 4.6 mm i.d. x 250 mm 5-μm (YMC Inc., Wilmington, NC, USA). The mobile phase was methanol (solvent A) and methyl tert-butyl ether (solvent B) at a flow rate of 1.0 ml/min. The gradient procedure was as follows: 1) Initial conditions 62% solvent A and 38% solvent B kept 27 min for column equilibration, 2) a 25 min linear gradient to 81.75% solvent B, 3) 2 min linear gradient to 100% solvent B, 4) 10 min with 100% solvent B for column cleaning. Column temperature was maintained at 30 °C. Standard solutions were prepared with all-trans-lycopene purchased from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were of HPLC grade. Before chemical analyses, the standard solutions and samples were filtered through Nalgene PTFE 0.22 μm filter. Retentate and permeate samples were collected in 7.4 ml amber glass vials and stored in the freezer at -80 °C before HPLC analysis.

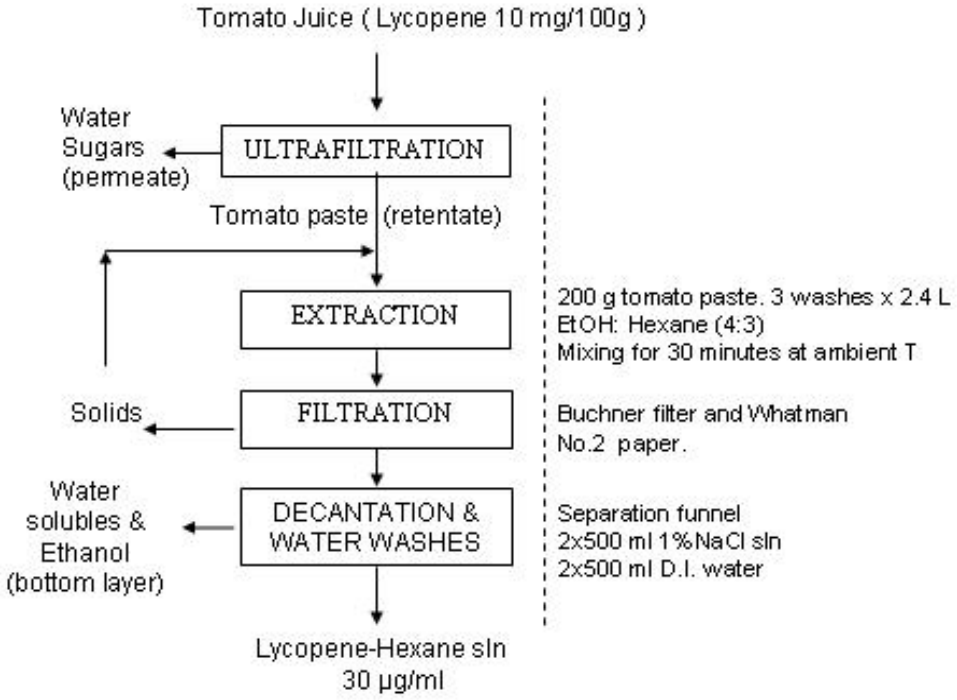

Figure 6.3 Schematic diagram for lycopene concentration and extraction.

Table 6.2- Physical properties for the solvents used in the experiments.
(Daubert and Danner 1989; Snyder 1974)

Solvent	Water (H_2O)	Ethanol (C_2H_5OH)	Hexane (C_6H_{14})
Molecular weight	18.02	46.07	86.18
Density (g/ml)	0.9980	0.7894	0.6594
Viscosity (mPa s)	1.002	1.078	0.326
Surface tension (mN/m)	72.75	22.32	17.9
Polarity index	10.2	4.3	0.1
Boiling point (ºC)	100	78.32	68.7
Water solubility (%w/w)	-	100	inmisc.
Dielectrical constant	78.3	24.3	1.9

6.3. Results and discussion

6.3.1. Membrane selection

To study the membrane characteristics, the performance of the membranes is interpreted from the solvent flux and solutes rejection. The following calculations are used for the experiments:

Flux (J) is defined as:

$$J(LMH) = \frac{\text{Volume of permeate (ml) x 3600 (s) x 1 (L)}}{\text{1000 (ml) x Time (s) x 1 (h) x 0.0126 } (m^2)} \quad (6.1)$$

Rejection (R) is defined as:

$$R(\%) = \left(1 - \frac{C_P}{C_R}\right) \times 100 \quad (6.2)$$

where C_p is the concentration of the solute in the permeate and C_R is the concentration of the solute in the retentate.

The volume concentration ratio (VCR) is defined as:

$$VCR = \frac{\text{Volume of Feed (L)}}{\text{Volume of retentate(L)}} = \frac{\text{Mass of Feed (g) / Density (g/L)}}{\text{Mass of Retentate (g) / Density (g/L)}} \quad (6.3)$$

The performance of the five membranes was evaluated by comparing rejection and flux under the same operating conditions. Initial screening was done at 26.7^{o}C and 200, 350, and 500 psig using lycopene-hexane extracts containing 20-30 µg/ml lycopene as the feed. Results are shown in Figures 6.4 and 6.5. Neither flux nor rejection were obtained for NF 45, because the preliminary conditioning showed that hexane flux could not be maintained due to short term dissolution of membrane and/or support/backing material. Membrane DS5-DK presented initial fluxes between 30 and 45 LMH but when pressure was maintained flux either decreased to a minimum (less than 1 LMH) or was stopped. This can be explained by an extensive swelling leading to pore constriction (higher membrane resistance) accompanied by membrane dissolution /disintegration. Membranes MPF 50, DS 7, and ES 404 showed flux increases with pressure, while rejection decreased or was maintained with pressure. ES 404 showed high flux, but low rejections. These results suggest that ES 404 membrane had probably swollen in the hexane which leads to pore dilatation and lower membrane resistance. This phenomenon is normally accompanied by long term membrane dissolution/disintegration. Membrane MPF 50 presented the better fluxes (61, 192, and 398 LMH respectively at 200, 350, and 500 psi TMP),

but rejections were low (29 to 31%) since the MWCO of the membrane (700 Da) is higher than the molecular weight of lycopene (536.85 Da). The best performance was shown by DS 7. Fluxes were 75, 143 and 174 LMH at 200, 350, and 500 psi TMP and rejections 73 to 76%. Therefore, this membrane was selected for subsequent experiments.

6.3.2 Flux model

Flux can be expressed in terms of a convective transport model:

$$J_v = \frac{L_p P}{\mu} \tag{6.4}$$

where J_v is the flux, L_p is the permeability coefficient of the membrane, P is the transmembrane pressure, and μ is the viscosity of the permeate. Membranes not affected by the solvent properties should give a linear plot of flux vs. 1/viscosity (Darcy's plot). A non-linear plot indicates the effect of solvent on membranes such as swelling of polymer and dilation of pores (Shukla 2000). For hydrophilic membranes, water is usually used for initial flux study and also for checking flux after cleaning. Hexane was used for testing the flux on the hydrophobic membranes DS 7 and MPF 50. Figure 6.6 shows the pressure effect on the hexane flux for these membranes. Flux increases linearly with an increase in TMP for MPF 50 membrane DS 7 membranes. The flux of pure hexane indicates the maximum flux achievable for the membrane. Solvents with higher molecular weight will have lower flux.

The permeability can be calculated by simplifying equation 6.4 as follows:

$$J_v = AP \tag{6.5}$$

where J_v is solvent flux, A is solvent permeability at a particular temperature, and P is the applied transmembrane pressure. Slope of the plot of flux vs. transmembrane pressure is the solvent permeability at a particular temperature. Hexane permeability at 26.7 ^{o}C was 44.4 LMH/Mpa for the DS 7 and 129.4 LMH/Mpa for the MPF 50.

6.3.3. Lycopene separation by membrane

The performance of DS7 membrane in separating lycopene from hexane solutions at various pressures (200 to 600 psi) was evaluated up to VCR 5. Considering that lycopene is susceptible to degradation by heat, light and oxygen, all the experiments in these figures were conducted at low temperatures (26.7 to 40 ^{o}C) with a lycopene concentration of 15 to 30 μg/ml .

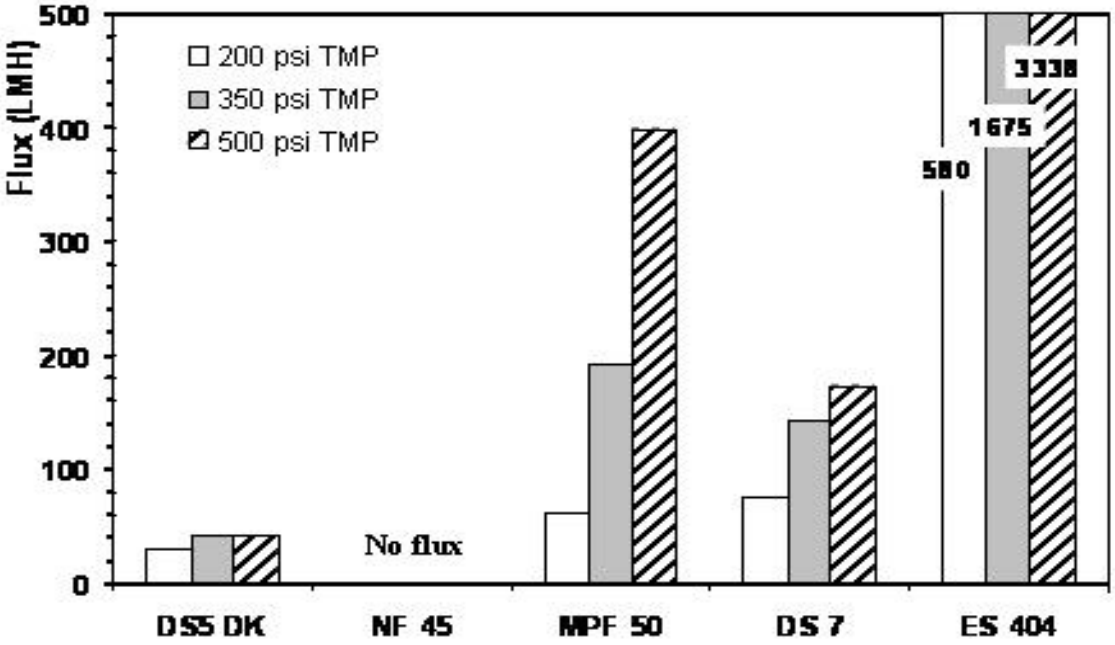

Figure 6.4 Pressure effect on flux for 5 membranes with lycopene-hexane extract. Concentration of lycopene at 30µg/mL and temperature at 26.7°C.

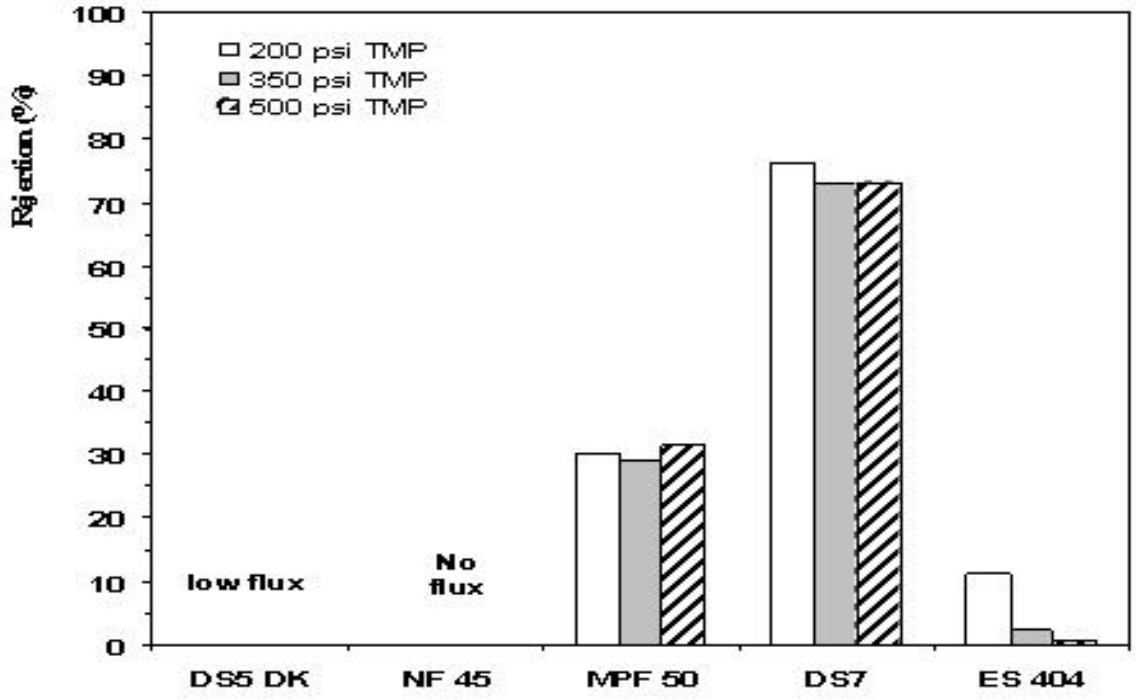

Figure 6.5 Pressure effect on rejection for 5 membranes with lycopene-hexane extract. Concentration of lycopene 30 µg/mL and temperature at 26.7°C

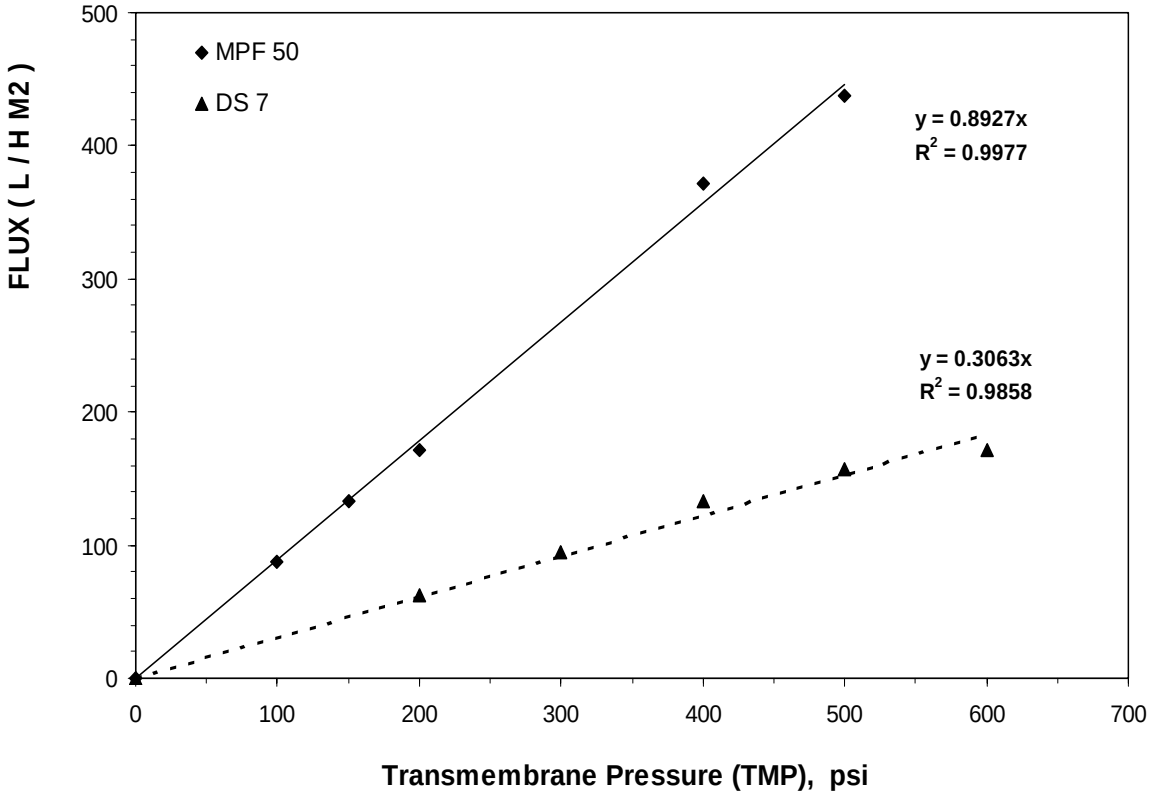

Figure 6.6. Effect of transmembrane pressure on hexane flux for DS 7 and MPF 50 membranes. Temperature 26.7 ^{o}C, feed velocity 240 l/h.

Figure 6.7 shows the typical lycopene concentration in retentate and permeate and the corresponding flux and rejection at 400 psi TMP. In general, the membrane was able to separate lycopene from hexane with a rejection of 64%-78% with an average of 72% depending on the process conditions. At VCR 5, the lycopene concentration went up from 17.5 µg/ml (the feed) to 43 µg/ml lycopene depending on the process conditions. This is an increase of up to 246% of the original concentration in the feed. The permeate initially contained 6 µg/ml lycopene, but then increased to 10.2 µg/ml at VCR 5. These results indicate that membrane separation of lycopene from tomato extracts is technically feasible. Additionally, the lycopene isomer pattern and the relative amounts were the same in lycopene-hexane-extracts, before and after membrane separation.

Low rejections lead to low yield of lycopene, according to the relationship as shown below (Cheryan 1998):

$$\text{Yield} = (\text{VCR})^{\sigma-1} \tag{6.6}$$

where VCR is the volume concentration factor and σ the rejection. Thus the yield of lycopene with a VCR 5 is 64% and for a VCR 10 is 52%. The low rejection of lycopene could be attributed to the shape/geometry of the molecules. As seen in Figure 2.1, lycopene consists of a long unsaturated aliphatic chain and 2 rings at the edges, which makes it possible for the molecule to pass through membranes with smaller nominal pore sizes (i.e.MWCO 400 Da for DS7 membrane) than the size of the molecule (536 Da). Low carotenes rejection of the DS 7 membrane with hexane-vegetable oil micelles was also reported by Koseoglu and others (1990), but the mechanism would be different since that membrane also allowed the passage of oil which has higher molecular weight than the carotene. The authors believed that the pigments formed a complex with phospholipids to form bigger molecules which were then rejected by the membrane.

6.3.3.1. Effect of transmembrane pressure

Pressure is the driving force of a membrane process as described in equations 6.4. If the feed contains a solute also, the equation becomes:

$$J_s = A\,(P - \Delta\pi) \tag{6.7}$$

where J_s is the flux, A the membrane permeability, P the trasmembrane pressure, and $\Delta\pi$ is the osmotic pressure differential due to the solute between the retentate and permeate side of the membrane.

For solutes with low molecular weight, osmotic pressure becomes important since it is directly proportional to the solute concentration and inversely proportional to the molecular weight of the solutes as approximated by the following van't Hoff equation (Cheryan 1998):

$$\pi = CRT/M \tag{6.8}$$

where π is osmotic pressure, C is solute concentration, R is gas constant, T is temperature and M is molecular weight of the solute.

The effect of pressure on permeate flux of lycopene hexane extracts are shown in Figure 6.8. Flux increased rapidly in the low pressure region and reached a steady state at pressures

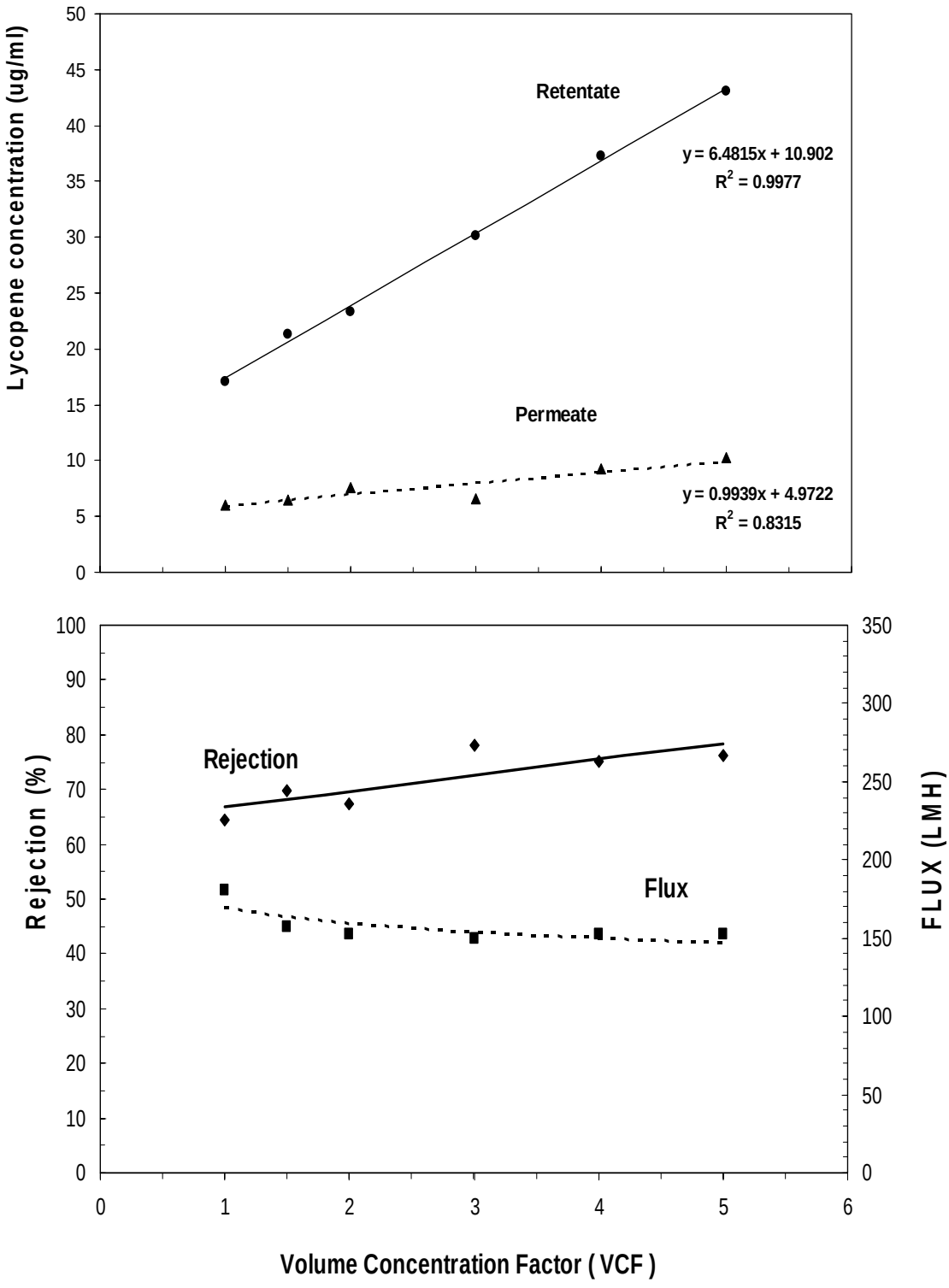

Figure 6.7 Performance of DS 7 membrane with lycopene hexane solution. Temperature 26.7 °C, TMP 400 psi, recirculation flow 240 l/h

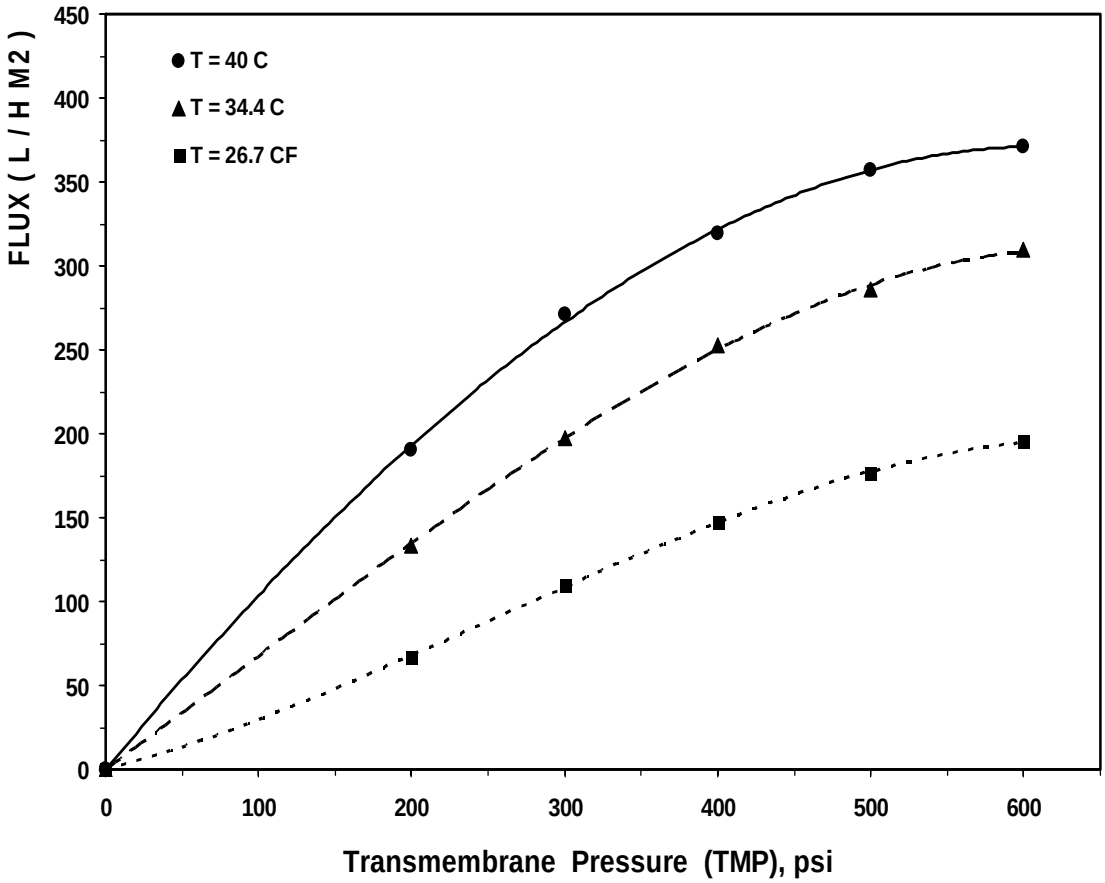

Figure 6.8 Effects of transmembrane pressure on flux at different temperatures. DS 7 membrane, and feed velocity 240 l/h.

higher than 400 psi. The fluxes obtained at 400psi and 500 psi were not statistically different.

The literature indicates that the lower pressure region is a pressure controlled region and the higher pressure region is a mass transfer controlled region. Concentration polarization leads to smaller incremental increases in flux as pressure increased until a gel layer is formed, at which point the flux shows no further increase with pressure. The flux at this point is called the Limiting Flux (Raman and others 1994). In order to extend the membrane life, 400 psi is thought to be the optimum pressure. The flux of 319 LMH was obtained at 400 psi, 40^{o}C, and feed velocity of 240 l/hr.

Effects of pressure on membrane selectivity are shown in Figure 6.9. A 69-71% lycopene rejection rate was maintained at 26.7^{o}C under all pressures . The rejection rates were

not significantly different under the various pressures. An increase in rejection was expected with increasing TMP due to concentration polarization but probably the gel layer was not formed because of the low solute concentration. Several models describe the phenomenon of increasing rejection at higher pressures (Cheryan and Nichols 1992). The solution-diffusion model describes solute rejection by the following equation:

$$R = \frac{A(\Delta P - \Delta\pi)}{A(\Delta P - \Delta\pi) + Bc} \quad (6.9)$$

where R is rejection, *A* is solvent flux , P is transmembrane pressure, π solute osmotic pressure, B is the rejecting capability of the membrane and c is concentration. According to this model, solvent flux increases linearly with pressure, reaching a maximum rejection of 1, while solute flux is independent of pressure. In practice, however, maximum rejection has never been achieved.

6.3.3.2. Effect of temperature

Temperature affects flux and rejection by altering the viscosity and diffusivity. Viscosity of the solvent decreases with an increase in temperature and thus flux increases as indicated by equation 6.4. High flux was obtained when the temperature increased (Figure 6.10), and followed a linear flux-temperature model. However the rejection decreased as temperature increased (Figure 6.10). This could be due to an increase in diffusivity allowing lycopene to diffuse at higher rate or due to swelling of the membrane at higher temperatures resulting in an increase in pore size. Because of the high decay of rejection with increasing the temperature and considering that lycopene is susceptible to degradation by heat, the lowest temperature (26.7 ^{o}C) was chosen as optimal. Low temperature also avoids significant vaporization of hexane during processing which has a boiling point of 60^{o}C. The flux obtained was 147.6 LMH at TMP 400 psi, temperature 26.7^{o}C, and feed velocity of 240 l/hr.

6.3.3.3. Effect of concentration

Figure 6.11 shows a flux decrease from 181 LMH at 17.1 µg/l ppm to 152 LMH at 23.3µg/l. Flux remained practically constant after increases in concentration from 23.3µg/l to 43.1µg/l.

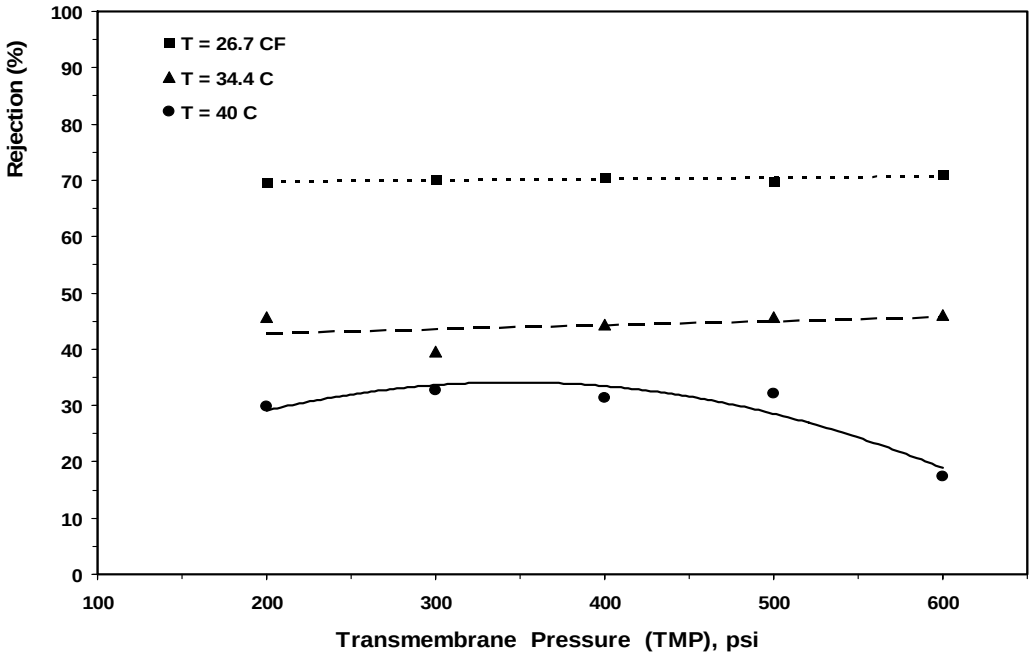

Figure 6.9 Effects of transmembrane pressure on lycopene rejection at different temperatures. DS 7 membrane, and feed velocity 240 l/h.

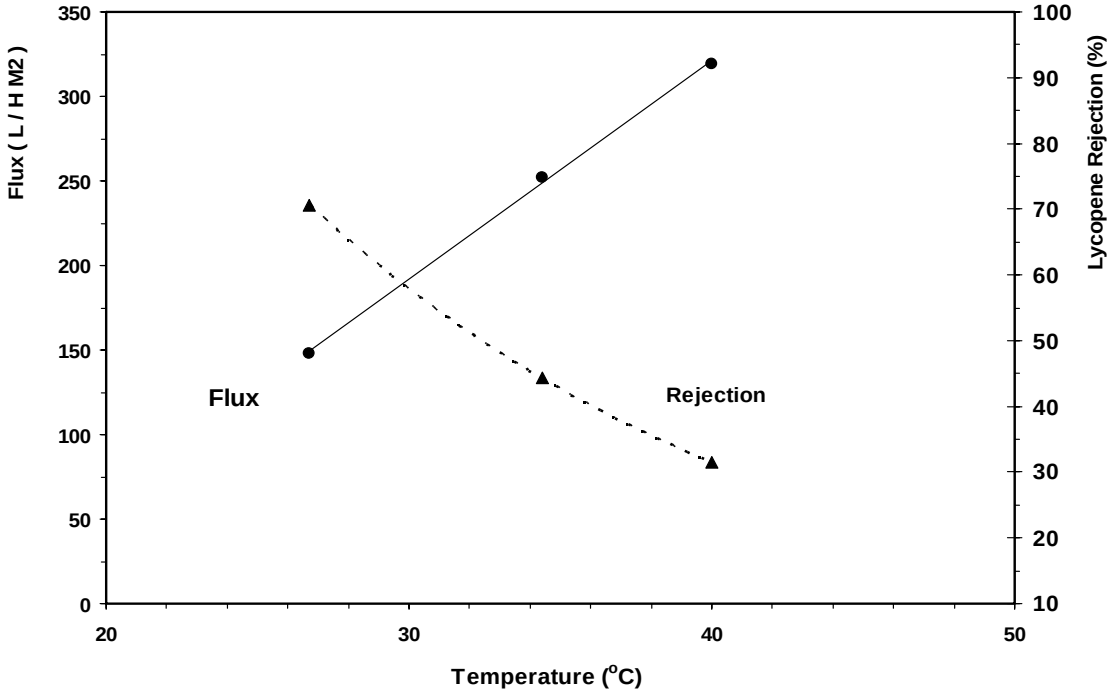

Figure 6.10 Effects of temperature on flux and lycopene rejection. DS 7 membrane, 400 psi TMP, feed velocity 240 l/h.

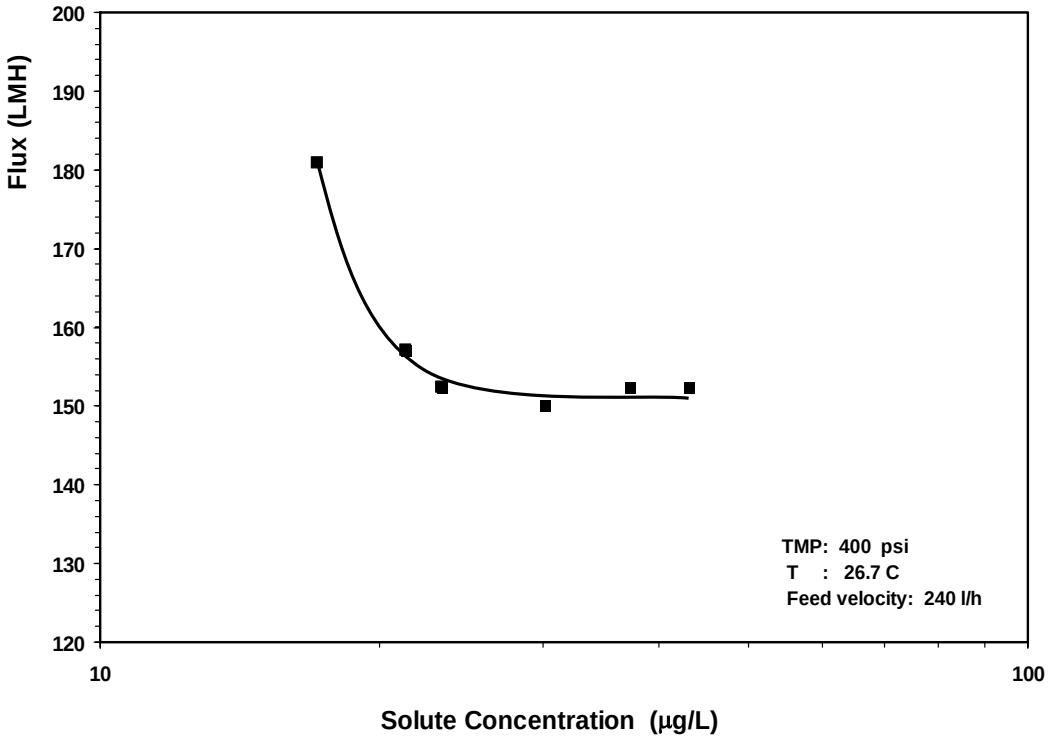

Figure 6.11 Effects of solute concentration on flux. DS 7 membrane, 400 psi TMP, feed velocity 240 l/h.

6.3.3.4. Lycopene concentration ratio

The relationship between solute concentration in retentate and VCR is given by the following equation (Cheryan 1998):

$$C_R = C_O(VCR)^{\sigma} \tag{6.10}$$

where C_R and C_o are concentration of solute in the retentate and feed, respectively, and σ is rejection. Figure 6.7 shows a plot of solute concentration in the retentate as a function of VCR. From equation 6.10, the concentration ratio of solute as a function of VCR can be expressed by the following equation (Cheryan 1998):

$$\log(SCR) = \sigma \log(VCR) \tag{6.11}$$

where SCR is solute concentration ratio (C_R/C_O) and σ is rejection. Plotting SCR as a function of VCR on a log-log scale will result in a straight line with rejection as the slope. This plot allows us to calculate rejection based on the retentate data only. Figure 6.12 shows such a plot obtained at 26.7°C and 400 psi with a feed concentration of 17.1µg/l lycopene. The slope of the plot was 0.3785 (R^2 = 0.9977). From these equations and the value of σ, we can determine the VCR to get the desired lycopene concentration. The final yield of lycopene at any VCR can be obtained using equation 6.6.

6.3.3.5. Process design and economics

Since the membranes did not reject 100% of the lycopene in the hexane extracts, a small quantity was escaping into the permeate. A multiple-stage process could be used to increase the recovery of lycopene; the permeate from the first stage NF could be fed into another NF system to recover more lycopene, and so on.

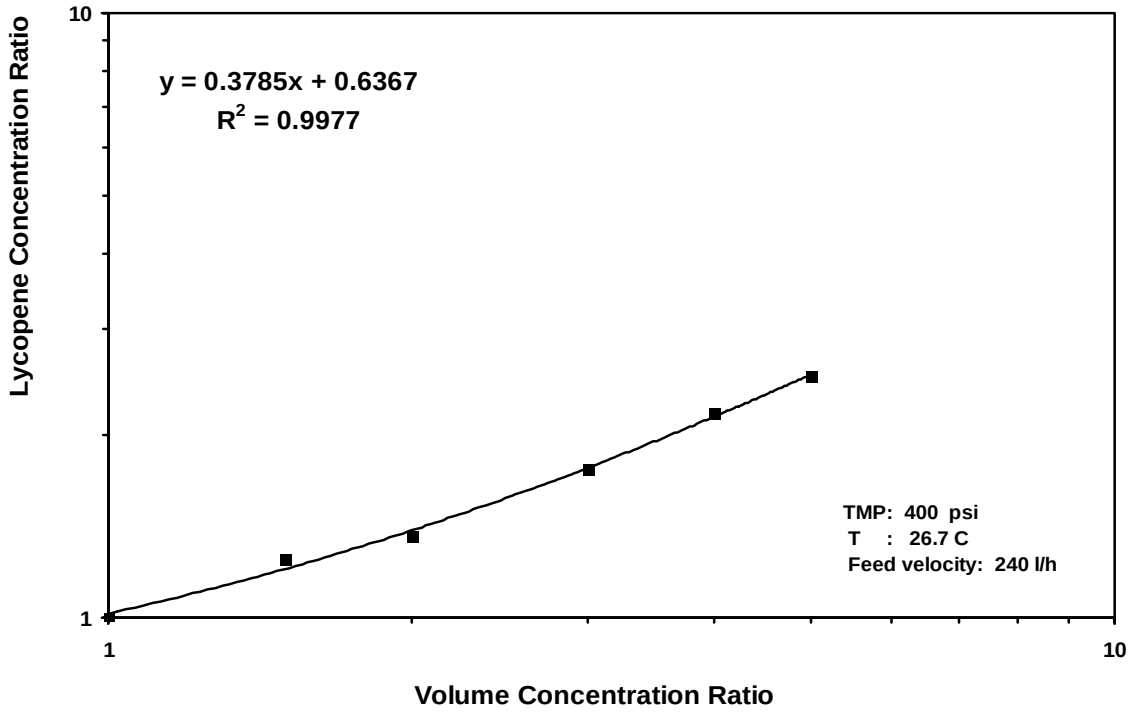

Figure 6.12 Plot of solute concentration ratio as a function of volume concentration ratio. DS 7 membrane, 400 psi TMP, T 26.7 °C, feed velocity 240 l/h.

A preliminary design of a system to recover lycopene from hexane extracts by multistage NF is shown in Figure 6.13 for a plant capacity of 1,000 kg/h of tomato paste. The temperature was assume to be 26.7 ^{o}C and transmembrane pressure was 2.75 MPa (400 psi). The design goal was the production of a retentate stream containing 5 times the concentration of the feed that would go to evaporation, and a permeate stream with a low level of lycopene that could be directly recycled to extraction. Table 6.3 summarizes the process design.

Flux for the design at different VCR was estimated using the following correlation:

$$\text{Flux (LMH)} = 169.8\ (\text{VCR})^{-0.0891} \qquad (6.12)$$

where VCR is the volume concentration ratio. Experimental data for the correlation is shown in Figure 6.7. Rejection was assumed 72% for all VCR as could be inferred from Figures 6.7 and 6.9. VCRs for stages 2 to 5 were estimated by trial and error using equation 6.11 with a C_R of 157.4 mg/l, and C_O calculated by mass balance from the previous stage. Yield for the stage was calculated using equation 6.6.

The feed to the membrane system would ideally be 15,000 l/h of lycopene-hexane extract, containing 30 mg/l lycopene. The first stage of the membrane system concentrates lycopene from 30 mg/l to 157.4 mg/l with a VCR of 10. The flow rate of permeate from the first stage is 13,500 l/h (8,910 kg/h) with 15.8 mg/l lycopene, which will be processed in the second membrane stage. A VCR of 24.3 in the second stage will result in the expected retentate concentration of 157.5 mg/l lycopene. By mass balance, permeate in the second stage will have 9.8 mg/l lycopene. The third stage would have to be operated to a VCR of 47.6, and permeate with 6.6 mg/l lycopene. For the fourth stage VCR was 82.1, and permeate concentration 4.7 mg/l lycopene. For the fifth stage VCR was 135, and permeate concentration 3.6 mg/l lycopene. It should be remembered that, while the permeate from the fifth stage goes directly back to extraction, the retentates from each stage will go to evaporation and then recycle to extraction. The membrane plant has been designed according to the methods suggested by Cheryan (1998). Since organic solvents are being processed, the membrane plant requires more rigorous construction than a water-based membrane plant. Thus the plant cost has been increased to \$750/m^2. Average flux was calculated from equation 6.12 and used to obtain the required membrane area in each stage. As shown in Table 6.3, the capital cost of a one-stage membrane

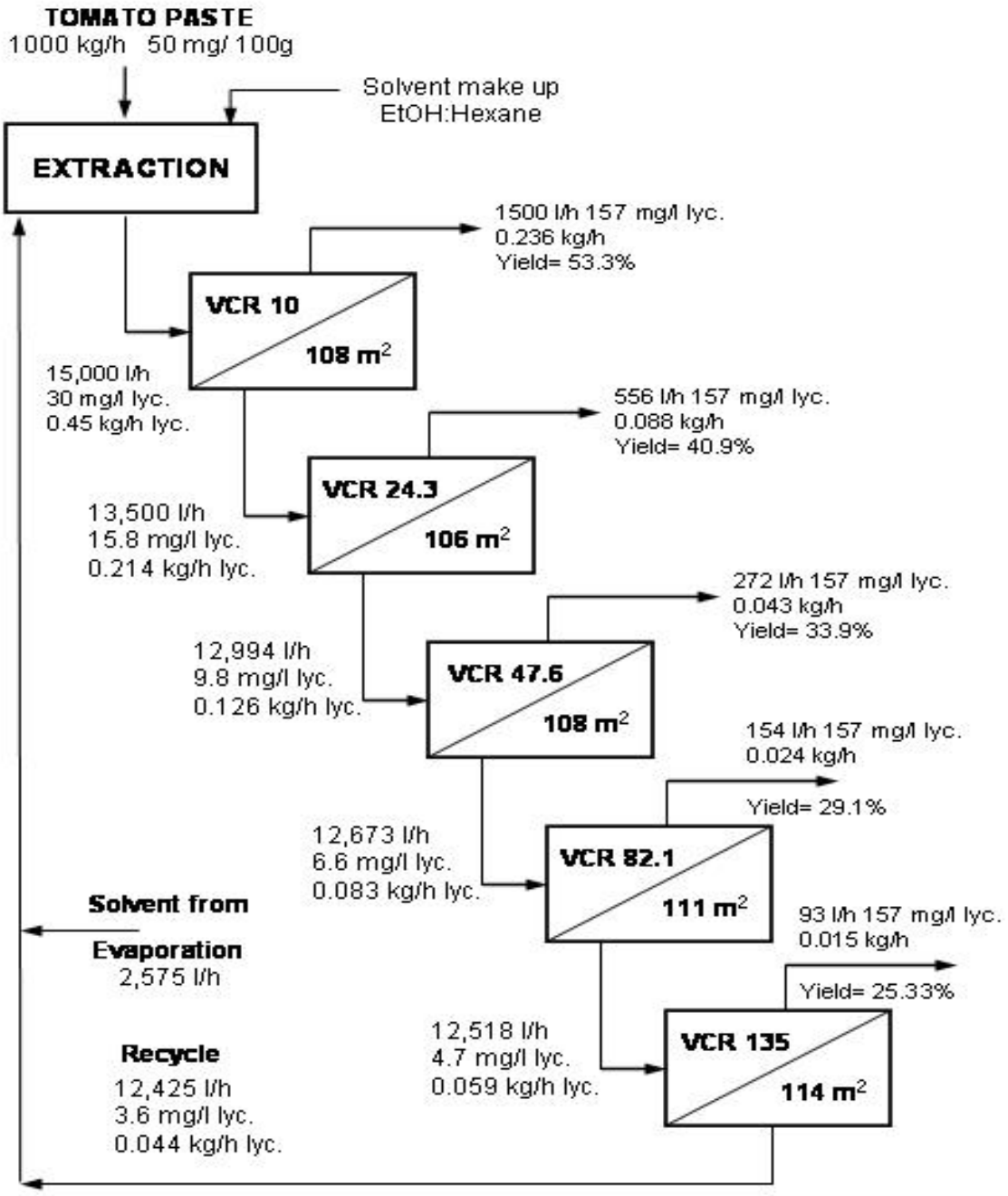

Figure 6.13 Mass balance of a multistage membrane system for recovery of lycopene from hexane extracts of tomato paste.

Table 6.3- Process design and cost for a multistage membrane system for recovery of lycopene from hexane extracts of tomato paste.

Stage	1	2	3	4	5	TOTAL
Feed, l/h	15,000	13,500	12,944	12,673	12,518	
Feed density	0.66	0.66	0.66	0.66	0.66	
Feed, kg/h	9,900	8,910	8,543	8,364	8,262	
VCR	10	24.3	47.6	82.1	135	
Rejection	0.72	0.72	0.72	0.72	0.72	
Retentate flow, L/h	1,500	556	272	154	93	2,575
Permeate flow, L/h	13,500	12,944	12,673	12,518	12,425	12,425
Flux, LMH	138	128	120	115	110	
Area of membrane, m2	108	106	108	111	114	546
Membrane cost, $/m2	750	750	750	750	750	750
Membrane plant cost, $	81,342	79,235	80,664	82,900	85,601	409,741
Depreciation, 15% of cap. cost, $/year	12,201	11,885	12,100	12,435	12,840	61,461
Operating cost, 0.3 of cap. cost, $/year	24,403	23,770	24,199	24,870	25,680	122,922
Lycopene conc. in feed, mg/L	30	15.8	9.8	6.6	4.7	
Lycopene conc. in retentate, mg/L	157.4	157.5	157.5	157.4	161.6	
Lycopene conc. in permeate, mg/L	15.8	9.8	6.6	4.7	3.6	
Lycopene produced by stage, kg/h	0.236	0.088	0.043	0.024	0.015	0.406
Lycopene produced by stage, kg/year	1417	525	257	146	90	2435
Lycopene produced by plant, kg/year	1417	1942	2199	2345	2435	
Lycopene Yield, %	52.5	40.9	33.9	29.1	25.3	
Lycopene Recovery, %	52.5	71.9	81.4	86.8	90.2	
Operating cost, $/kg	17.22	24.80	32.91	41.47	50.49	50.49
Annual income, $/year [a]	4,324,623	5,927,308	6,711,573	7,156,555	7,431,012	7,431,012
Annual net income, $/year	4,288,019	5,867,250	6,627,101	7,046,878	7,295,249	7,295,249
Value gain, ($ lycopene/$ oper.cost)	175.72	121.80	91.57	72.47	59.35	59.35

[a] Assumed lycopene price = 3,052 $/kg.

plant is about $81,342, equivalent to about $57/kg per hour of tomato paste capacity. A 5-stage plant will cost $168/kg per hour of tomato paste capacity. The annual operating cost is based on depreciation, membrane replacement, cleaning, labor and maintenance as discussed by Cheryan (1998). Operating cost varies from $17.22 to $50.49 per kg of lycopene recovered, depending on the number of stages.

Although lycopene recovery increases from 52.5% with one stage to 90.2% with five stages (Table 6.3), the amount of hexane recycled from the membrane system decreases slightly, resulting in more hexane to be evaporated (1500 l/h with one stage versus 2575 l/h with five stages). In addition, the capital and annual operating cost of the membrane plant increases with the number of stages. However, the expected high value of the lycopene recovered compensates for the additional stages. The lycopene recovered in a one-stage plant is 1417 kg per year, worth over $ 4 million (assuming a lycopene value of $ 3052/kg as estimated in Table 6.4).

Table 6.4- Price of lycopene tablets/capsules.

Company	Presentation	Lycopene content	Price		Selling Price [1]
	(tablets/bottle)	(mg/tablet)	($/bottle)	($/kg)	($/kg)
Twinlab	30	10	9.77	32,567	3,257
Twinlab	60	10	19.99	33,317	3,332
Stay-well	100	5	9.95	19,900	1,990
Protectamins	60	5	11.99	39,967	3,997
Phyto Pharmica	45	10	7.46	16,578	1,658
Healthy Origins [2]	30	15	19.99	44,422	4,442
Swanson [2]	60	10	8.09	13,483	1,348
Natural Factors [2]	60	10	17.00	28,333	2,833
ClubNatural [2]	60	10	21.99	36,650	3,665
Nature's Way [2]	60	5	11.99	39,967	3,997
			Average	30,518	3,052

(1) Estimated price for selling at plant. Assumes 1/10 of tablet/capsules retail price
(2) Use lycopene "Lyco-O-mato" from Lyco-Red

In terms of value gained, since the annual operating cost for one stage is $24,403, the membrane plant is worth $175.72 per $ of operating cost. As shown in Table 6.3, although the net value gained decreases with more stages, a five-stage plant is still an attractive $59.35 per $ of operating cost.

6.4. Conclusions

The results shown in this study indicate that NF separation of lycopene from hexane extracts is technically feasible. Previous manufacturing processes require a separation step such as conventional distillation or rotary evaporator which consumed a lot of energy and increased the degradation of lycopene at high temperature. A new economically viable process to purify the lycopene from hexane extracts is developed with NF stages in series. The process is low in energy consumption and the operating conditions are mild enough to minimize damage to lycopene. Applications of membranes in non-aqueous solvents have been limited because of the poor stability and poor understanding of flux model of polymeric membranes in organic solvents. The MWCO in water provided by the manufacturers is insufficient to characterize the membrane in organic solvents. Five polymeric membranes were screened with lycopene-hexane extracts. Two of them, Osmonics' DS 7 and Koch's MPF 50 were suitable for the lycopene-hexane extraction studies. DS 7 manufactured by Osmonics-Desal was the best in terms of flux, rejection and stability.

The optimum operating conditions for concentrating lycopene hexane by NF were 26.7°C and 400 psi. The DS 7 membrane resulted in an average flux and rejection of 152 LMH and 72% respectively at 26.7°C and 400 psi when concentrating lycopene in hexane from 17.1µg/ml to VCR 5. A multi-stage membrane processing of lycopene-hexane extracts with an initial concentration of 30 mg/l could require 5 stages to produce a lycopene concentrate of 157mg/l with recovery of 90.2%. Economic calculations show that the industrial application of membrane technology for recovering lycopene is promising and profitable. More research should be performed specially on the engineering aspects of the process using cross flow membrane system at a larger scale.

7. SUMMARY AND CONCLUSIONS

Consumer demand for healthful food products provides an opportunity to develop an market for food and pharmaceutical-grade lycopene products. Currently available methods of manufacturing are either inefficient or require large amounts of energy. Because of its health benefits and high cost processing, pure lycopene and the nutraceuticals and functional foods containing it, are high price products. The membrane process developed in this work provides a more environmentally friendly extraction and purification procedure on an industrial scale with minimal loss of bioactivity which is highly desirable for the food, feed, cosmetic, and pharmaceutical industries. This process is also an alternative to the industry in its search for lower operating cost and higher yields because is a proven low-energy, low temperature method of separation.

The membrane separation of bioactive lycopene from tomato juice was proposed in three steps: Membrane ultrafiltration concentration, solvent extraction and nanofiltration separation.

In the ultrafiltration study, three polymeric membranes (PCI's FP 200, PCI FP 100, and PCI ES 404) were screened for lycopene concentration in a cross-flow pilot plant-scale membrane unit with tomato juice containing 10-11 mg lycopene/100g of tomato juice. Lycopene was not detected in the permeate stream with the analysis performed, indicating a total rejection of lycopene for all three membranes tested. PCI's FP 200 membrane was the best in terms of flux with average of 155 LMH at 60°C and 50 psi TMP for a 4.5X concentration process. The tomato concentrate obtained as retentate, contained 51.7 mg lycopene/100g sample.

In the solvent extraction study, five solvents (ethanol, ethyl acetate, tetrahydrofuran, acetone, and isopropanol) were tested at ambient temperature (21-23 °C) as cosolvents with hexane for extraction of lycopene from the tomato concentrate obtained by UF. Ethanol:hexane (4:3) resulted in the highest recovery. Multiple extractions of the same tomato concentrate with fresh ethanol:hexane resulted in an all-trans-lycopene yield of 91% after 3 extractions at a solvent-to-solids ratio of 45 ml per g. Total recovery of carotenoids in the tomato paste was 58.8 mg/100g where all-trans-lycopene counted for 87.9%, β-carotene for 4.9%, 13-cis-lycopene for 3.2%, 7-cis-lycopene for 2.2%, and unidentified lycopene cis-isomers for 1.7%.

The results shown in the last part of this work indicate that NF separation of lycopene from hexane extracts is technically feasible. A new economically viable process to purify the lycopene from hexane extracts is developed with NF stages in series. The process is low in

energy consumption and the operating conditions are mild enough to minimize damage to lycopene. Applications of membranes in non-aqueous solvents have been limited because of the poor stability and poor understanding of flux model of polymeric membranes in organic solvents. The MWCO in water provided by the manufacturers is insufficient to characterize the membrane in organic solvents. Five polymeric membranes were screened with lycopene-hexane extracts. Two of them, Osmonics' DS 7 and Koch's MPF 50 were suitable for processing the lycopene-hexane extract. The DS 7 manufactured by Osmonics-Desal was the best in terms of flux, rejection and stability. The optimum operating conditions for concentrating lycopene hexane by NF were 26.7°C and 400 psi. The DS 7 membrane resulted in an average flux and rejection of 152 LMH and 72% respectively at 26.7°C and 400 psi when concentrating lycopene in hexane from 17.1 μg/ml to VCR 5. A multi-stage membrane processing of lycopene-hexane extracts with an initial concentration of 30 mg/l could require 5 stages to produce a lycopene concentrate of 157 mg/l with recovery of 90.2%. Economic calculation shows that the industrial application of membrane technology for recovering lycopene is promising and profitable.

More research should be performed specially on the engineering aspects of the process using cross flow membrane system at pilot plant scale. Membrane fouling and cleaning, and long time solvent stability should be further studied for both, ultrafiltration and nanofiltration steps. Effect of lower temperatures on rejection of lycopene-hexane extracts should be also explored.

Further studies focused in optimization of the solvent extraction (higher temperatures and lower solvent to solids ratio) should consider use of boiling ethanol and other GRAS solvents because of coming government regulations against hexane use in the industry.

REFERENCES

Atsushi S, Shoichi T, Kouichirou T, Masaji K, Tadaaki W, Eiji I, inventors; Kikkoman Corp., Nippon del Monte Corp., assignees. 2002 May 8. Lycopene composition, method of producing the same and use thereof. Japanese patent JP2002125619.

Ausich RL and Sanders DJ, inventors; Kemin Foods LC, assignee. 1999 Jan 12. Process for the isolation and purification of lycopene crystals. US patent 5,858,700.

Batieha A.M., Armenian H.K., Norkus E.P., Morris J.S., Spate V.E., and Comstock G.W. 1993. Serum micronutrients and subsequent risk of cervical cancer in a population-base nested case-control study. Cancer Epidemiol. Biomarkers Prev. 2:335-339.

Bhanushali D, Kloos S, Kurth C, and Bhattacharyya D. 2001. Performance of solvent-resistant membranes for non-aqueous systems: solvent permeation results and modeling. Journal of Membrane Science 189:1-21.

Black LE, inventor; Exxon Research and Engineering Co, assignee. 1991 Apr 10. Interfacially polymerized membranes, and reverse osmosis separation of organic solvent solutions using them. European patent application EP 0421676A1.

Blanc P, Larbot A, Palmeri J, Lopez M, and Cot L. 1998. Hafnia ceramic nanofiltration membranes. Part I: Preparation and characterization. Journal of Membrane Science 149:151-161.

Beerh OP, and Siddappa GS. 1959. A rapid spectrophotometric method for the detection and estimation of adulterants in tomato ketchup. Food Technol. 7:414-418.

Bemberis I and Neeky K. 1986. Ultrafiltration as a competitive unit process. AIChE Symp. Ser. 250:65-77.

Benet LZ and Shiner LB. 1985. Pharmacodynamics: the dynamics of drug absorption, distribution and elimination. In: Goodman Gilman A., Goodman L.S., Rall T.W., and Murad F. Pharmacological Basis of Therapeutics. 7th ed. New York: Macmillan. p 345-484.

Benfer S, Popp U, Richter H, Siewert C, and Tomandl G. 2001. Development and characterization of ceramic nanofiltration membranes. Separation and Purification Technology 22:231-237.

Benthin P, Danz H, and Hamburger M. 1999. Pressurized liquid extraction of medicinal plants. Journal of Chromatography A 835:211-219.

Bitter JGA, Haan JP, and Rijkens H.C. 1989. Solvent recovery using membranes in the lube oil dewaxing process. AIChE Symp. Ser. 85:98-100.

Bjelke E. 1974. Case-control study in Norway. Scand. J. Gastroenerol. 9:42-49.

Böhm V. 2001. Use of column temperature to optimize carotenoid isomer separation by C_{30} high performance liquid chromatography. Journal of Separation Science 24:955-959.

Boileau AC, Merchen NR, Wasson K, Atkinson CA, and Erdman JW. 1999. Cis-lycopene is more bioavailable than trans-lycopene in vitro and in vivo in lymph-cannulated ferrets. J. Nutr. 129:1176-1181.

Bombardelli E, Morazzoni P, Seghizzi R, inventors; Indena S.p.A., assignee. 1999 Apr 27. Process for the extraction of lycopene using phospholipids in the extraction medium. US patent 5,897,866.

Bortlik K, Mortezavi L, Saucy F, inventors; Societe des produits Nestle S.A. 2001 May 30. A process for the extraction of lycopene. European patent EP 1103579.

Boskovic F, Backhaus RA, and Camara B. 1979. Induction and control of chromoplast-specific carotenoid genes by oxidative stress. J. Biol. Chem. 273:30651-30659.

Breitenbach J, Braun G, Steiger S, and Sandmann G. 2001. Chromatographic performance on a C30-bonded stationary phase of monohydroxycarotenoids with variable chain length or degree of desaturation and of lycopene isomers synthesized by various carotene desaturases. Journal of Chromatography A 936:59-69.

Bruno RS, Wildman REC. 2001. Lycopene: source, properties and nutraceutical potential. In: Wildman REC editor. Handbook of Nutraceuticals and Functional Foods.Boca Raton, FL: CRC Press. p 157-168.

Cano MP, Ancos B, Lobo G, Monreal M, and De Ancos B. 1996. Effect of freezing and canning of papaya slices on their carotenoid composition. Z. Lebensmittel Unters Forsch. 202(4):279-284.

Cheryan M. 1998. Ultrafiltration and Microfiltration Handbook. 2nd ed. Lancaster, PA: Technomic Publishing Co. 527 p.

Cheryan M and Rajagopalan, N. 1998. Membrane processing of oily streams. Wastewater treatment and waste reduction. Journal of Membrane Science 151:13-28.

Cheryan M and Alvarez JR. 1995. Food and beverage industry applications. In: Noble RD and Stern SA, editors. Membrane Separations Technology. Principles and Applications. New York: Elsevier Science. p 417-465.

Clinton SK. 1998. Lycopene: chemistry, biology and implications for human health and disease. Nutr. Rev. 56(2):35-51.

Clinton SK, Emenhiser C, Schwartz SJ, Bostwick D.G., Williams A.W., Moore B.J., and Erdman J.W. 1996. Cis-trans lycopene isomers, carotenoids, and retinol in the human prostate. Cancer Epidemiol. Biomarkers Prev. 5:823-833.

Cole ER and Kapur NS. 1957b. The stability of lycopene. II. Oxidation during heating of tomato pulps. J. Sci. Food Agric. 8:366-368.

Cole E.R. and Kapur N.S. 1957a. The stability of lycopene. I. Degradation by oxygen. J. Sci. Food Agric. 8:360-365.

Cook-Mozaffari PJ, Azordegan F, and Day NE. 1979. Esophageal cancer studies in the Caspian Littoral of Iran: results of a case- control study. Brit. J. Cancer 39:292-309.

Conn PF, Lambert C, Land EJ, Schalch W, and Truscott TG. 1992. Free radicals. Res. Commun. 16:401-408.

Conn PF, Shalch W, and Truscott TG. 1991. The singlet oxygen and carotenoid interaction. J. Photochem. Photobiol. B. Biol. 11: 41-47.

Craft NE. 1992. Carotenoid reverse-phase high-performance liquid chromatography methods. Reference compendium. Meth. Enzymol. 213:185-205.

Daubert TE and Danner RP. 1989. Physical and Thermodynamic Properties of Pure Chemicals: Data Compilation. New York: Hemisphere Pub. Corp. 540 p.

Diaz MN, Frei B, Vita JA, and Keany JF. 1997. Antioxidants and atherosclerotic heart disease. New Eng. J. Med. 337: 408-416.

Di Mascio P., Kaiser S., and Sies H. 1989. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch. Biochem. Biophys. 274:532-538.

D'Souza MC, Singha A, and Ingle M. 1992. Lycopene concentration of tomato fruit can be estimated from chromaticity values. Hort. Science 27:465-466.

Emenhiser C, Sander Lane C, Schwartz SJ. 1995. Capability of a polymeric C30 stationary phase to resolve cis-trans carotenoid isomers in reverse-phase liquid chromatography. Journal of Chromatography A 707:205-216.

Epler KS, Sander LC, Ziegler RG, Wise SA, and Craft NE. 1992 Evaluation of reverse-phase liquid chromatographic columns for recovery and selectivity of selected carotenoids. Journal of Chromatography. 595:89-101.

Estrella del Castro A, Bernasconi E, Collados A, Esteban M, Gonzalez E, inventors; Vitatene SA, assignee. 2002 May 2. Process for producing lycopene. European patent EP 1201762.

Farnand BA, Talbot FDF, Matsuura T, and Sourirajan S. 1984. Reverse osmosis separations of some inorganic solutes in ethanol solutions using cellulose acetate membranes. Sep. Sci. Technol. 19(1): 33-50.

Ferruzi MG, Nguyen ML, Sander LC, Rock CL, Schwartz SJ. 2001. Analysis of lycopene geometrical isomers in biological microsamples by liquid chromatography with coulometric array detection. Journal of Chromatography B 760:289-299.

Franceschi S, Bidoli E, La Vecchia C, Talamini R, D'Avanzo B, and Negri E. 1994. Tomatoes and risk of digestive-tract cancers. Intl. J. Cancer 59(2):181-184.

Gartner C, Stahl W, and Sies H. 1997. Lycopene is more bioavailable from tomato paste than from fresh tomatoes. Am. J. Clin. Nutr. 66:116-122.

Gester H. 1997. The potential role of lycopene for human health. J. Am. Col. Nutr. 16(2):109-126.

Giovannucci E., Ascherio A, Rimm EB, Stampfer M., Colditz GA, and Willett WC. 1995. Intake of carotenoids and retinol in relationship to risk of prostate cancer. J. Natl. Cancer Inst. 87:1767-1776.

Golemme G and Drioli E. 1996. Polyphosphazene membrane separations-review. Journal of Inorganic and Organomet. Polymers 6(4):341-365.

Gross J. 1991. Pigments in Vegetables: Chlorophylls and Carotenoids. New York: Van Nostrand Reinhold. 351 p.

Gross J. 1987. Pigments in Fruits. London: Academic Press. 303 p.

Guizard C, Julbe A, and Ayral A. 2000. Current status of the development of microporous or nanoporous ceramic membranes. Industrial Ceramics 20(1):22-25.

Hafez MM and Koenitzer BA, inventors; Exxon Research and Engineering Co., assignee. 1985 Apr 17. Method of preswelling of regenerated cellulose membranes for organic separations and the use thereof. European patent EP 0137698

Hakala SH and Heinonen IM. 1994. Chromatographic purification of natural lycopene. J. Agric. Food Chem. 42:1314-1316.

Hart DJ and Scott KJ. 1995. Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. Food Chem. 54:101-111.

Hartal D, Raveh Y, Wolf A, inventors; 1999 Oct 12. Stable lycopene concentrates and process for their preparation. US patent 5,965,183.

Hengartner U, Bernhard K, Mayer K, Englert G, and Glinz E. 1992. Synthesis, isolation and NMS-spectroscopic characterization of fourteen (Z)-isomers of lycopene and of some acetylenic didehydro- and tetrahydrolycopenes. Helv. Chim. Acta 75:1848-1865.

Hestekin JA, Smothers CN, and Bhattacharyya D. 2001. Nanofiltration of charged organic molecules in aqueous and non-aqueous solvents: separation results and mechanisms. In: Nunes SP and Peinemann KV editors. Membrane Technology. Weinheim, Germany: Wiley-VCH. pp. 173-190.

Hestekin JA, Smothers CN, and Bhattacharyya D, Ghorpade A, and Hannah R. 1999. Nanofiltration for removal of organics from aqueous and organic solvent streams. Paper presented at the AIChE Annual Meeting, Fourth Topical Conference on Separations Science and Technology, Dallas, Texas, October 31-November 5, 1999. Available at the Linda Hall Library of Science, Engineering & Technology (www.lindahall.org), call no. 99An37i. p.1-5.

Hussein L and El-Tohamy M. 1990. Vitamin A potency of carrot and spinach carotenes in human metabolic studies. Int. J. Vit. Nutr. Res. 60:229-235.

Ishida BK, Ma J, and Chan B. 2001. A simple, rapid method for HPLC analysis of lycopene Isomers. Phytochemical Analysis 12:194-198.

Iwama A. 1987. New process for purifying soybean oil by membrane separation and economic evaluation of the process. J. Am. Oil Chem. Soc. 64:1258.

Jackson MJ. 1997. The assessment of bioavailibility of micronutrients: introduction. Eur. J. Clin. Nutr. 51:S1-S2.

Jarvinen R, Knekt P, Seppanen R, and Teppo L. 1997. Diet and breast cancer risk in a cohort of Finnish women. Cancer Lett. 114:251-253.

Kale V, Katikaneni SPR, and Cheryan M. 1999. Deacidifying rice bran oil by solvent extraction and membrane technology. J. Am. Oil Chem. Soc. 76:723-727.

Khachik F, Beecher GR., and Smith JC. 1995. Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. J. Cell Biochem. Suppl. 22:236-246.

Khachik F, Goli MB, Beecher GR, Holden J, Lusby WR, Tenorio MD, and Barrera MR. 1992a. Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. J. Agric. Food Chem. 40:390-398.

Khachik F, Beecher GR, Lusby WR, and Smith JC. 1992b. Separation and identification of carotenoids and their oxidation products in the extracts of human plasma. Anal. Chem. 64:2111-2122.

Kimura M, Rodriguez-Amaya DB, and Godoy HT. 1990 Assessment of the saponification step in the quantitative determination of carotenoids and provitamins A. Food Chem. 35:187-195.

Klein E, Smith JK. 1972. The use of solubility parameters for solvent selection in asymmetric membrane formation. In*:* H. K. Lonsdale and H. E. Podall, editors. Reverse Osmosis Membrane Research. New York: Plenum Press. 503 p.

Koenhen DM and Tinnemans AHA, inventors; 1992. A semipermeable composite membrane, a process for the manufacture thereof, as well as application of such membranes for the separation of components in an organic liquid phase or in the vapor phase. US patent 5,274,047.

Koops GH, Yamada S, and Nakao SI. 2001. Separation of linear hydrocarbons and carboxylic acids from ethanol and hexane solutions by reverse osmosis. Journal of Membrane Science 189:24-254.

Köseoğlu SS, Lawhon JT, and Lusas EW. 1990. Membrane processing of crude vegetable oils: pilot plant scale removal of solvents from oil miscellas. J. Am. Oil Chem. Soc. 67:315-322.

Krinsky NI, Russet MD, Handeman GJ, and Snodderly DM. 1990. Structural and geometrical isomers of carotenoids in human plasma. J. Nutr. 120:1654-1662.

Kulkarni SS, Edward WF, and Li NN. 1992. Ultrafiltration. In: Winston WS and Sirkar K.K. Membrane Handbook. New York: Van Nostrand Reinhold. p 393-400.

Landers GM and Olson JA. 1986. Absence of isomerization of retinyl palmitate, retinol, and retinal in chlorinated and unchlorinated solvents under gold light. J. assn. Offic. Anal. Chem. 69:50-55.

Lencki RW and Williams S. 1995. Effect of non-aqueous solvents on the flux behavior of ultrafiltration membranes. Journal of Membrane Science 101:43-51.

Lessin W, Catigani G, and Schwartz SJ. 1997. Quantification of cis-trans isomers of provitamin A carotenoids in fresh and processed fruits and vegetables. J. Agric. Food Chem. 45:3728-3732.

Levy J, Bisin E, Feldman B, Giat Y, Minster A, Danilenko M, and Sharoni Y. 1995. Lycopene is a more potent inhibitor of human cancer cell proliferation the either α-carotene or β-carotene. Nutr. Cancer 24:257-266.

Luthra SS, Yang X, Freitas dos Santos LM, White LS, and Livingston AG. 2001. Phase-transfer catalyst separation and re-use by solvent resistant nanofiltration membranes. Chemical Communications 16:1468-1469.

Machado DR, Hasson D, and Semiat R. 1999. Effect of solvent properties on permeate flow through nanofiltration membranes. Part I: Investigation of parameters affecting solvent flux. Journal of Membrane Science 163:93-102.

Madley R.H. 2001. Reviving the antioxidant market. Nutraceuticals World 4 (3):42-52.

Manguels AR, Holden JM, Beecher GR, Forman MR, and Lanza E. 1993. Carotenoids in fruits and vegetables: and evaluation of analytic data. J. Am. Diet. Assoc. 93:284-296.

Merlo CA, Rose WW, Petersen LD, White EM, and Nicholson JA. 1986. Hyperfiltration of tomato juice. Pilot-scale high temperature testing. J. Food Sci. 51:403-407.

Meyer K, inventor; Hoffman-La Roche Inc., assignee. 1993 May 4. Method for the manufacture of carotenoids and the novel intermediates. US patent 5,208,381.

Meyer K, inventor; Hoffman-La Roche Inc., assignee. 1992 Nov 24. Method for the manufacture of carotenoids and the novel intermediates. US patent 5,166,445.

Miller NJ, Sampson J, Candeias LP, Bramley PM, Rice-Evans CA. 1996. Antioxidant activities of carotenes and xanthophylls. FEBS Lett. 384:204-242.

Musale DA and Kumar A. 2000. Solvent and pH resistance of surface cross linked chitosan/ poly(acrylonitrile) composite nanofiltration membranes. J. Appl. Poly. Sci. 77:1782-1793.

Nguyen ML and Schwartz SJ. 1999. Lycopene: chemical and biological properties. Food Technology 53(2):38-45

Nguyen ML and Schwartz SJ. 1998. Lycopene stability during food processing. Proc. Soc. Exp. Biol. Med. 218:101-105.

Nonomura AM, inventor; Microbio Resources Inc., assignee. 1987 Dec 15. Naturally-derived carotene/oil composition. U.S. patent 4,713,398.

Nonomura AM, inventor; Microbio Resources Inc., assignee. 1987 Jul 14. Process for producing a naturally-derived carotene/oil composition by direct extraction from algae. U.S. patent 4,680,314.

Nunes SP and Peinemann KV. 2001. Membrane Technology. Weinheim, Germany: Wiley-VCH. 314 p.

Nwuha V. 2000. Novel studies on membrane extraction of bioactive components of green tea in organic solvents: Part I. J. of Food Engineering 44(4):233-238.

Ojima F, Sakamoto H, Ishiguro Y, and Terao J. 1993. Consumption of carotenoids in photosensitized oxidation of human plasma and plasma low-density lipoprotein. Free Radic. Biol. Med. 15: 377-384.

Paulson DJ. 1995. Membranes, the finest filtration. Filtration News. Available from Osmonics (www.osmonics.com/products/page698.htm). Accessed May 11 2003.

Pepper D, Orchar ACJ, and Merry AJ. 1985. Concentration of tomato juice and other fruit juices by reverse osmosis. Desalinization 53:157-166.

Potischman N, Hoover RN, Brinton LA, Swanson CA, Herrero R, Tenori F, de Britton RC, Gaitan E, and Reeves WC. 1994. The relations between cervical cancer and serological markers of nutritional status. Nutr. Cancer 21(3):193-201.

Raman LP, Cheryan M, and Rajagopalan N. 1996a. Solvent recovery and partial deacidification of vegetable oils by membrane technology. Fette Wissenschaft Technologie 98(1):10-14.

Raman, LP, Cheryan M, and Rajagopalan N. 1996b. Deacidification of soybean oil by membrane technology. J. Am. Oil Chem. Soc. 73:219-224.

Ramon JM, Serra L, Cerdo C, and Oromi J. 1993. Dietary factors and gastric cancer risk. Cancer 71:1731-1735.

Reddy KK, Kawakatsu T, Snape JB, and Nakajima M. 1996. Membrane concentration and separation of L-aspartic acid and L-phenylalanine derivates in organic solvents. Separation Science and Technology 31(8):1161-1178.

Ribaya-Mercado JD, Garmyn M, Gilchrest BA, and Russel RM. 1995. Skin lycopene is destroyed preferentially over β-carotene during ultraviolet irradiation in humans. J. Nutr. 125:1854-1859.

Rouseff R, Raley L, and Hofsommer HJ. 1996. Application of diode array detection with a C30 reversed phase column for the separation and identification of saponified orange juice carotenoids. J. Agric. Food Chem. 44:2176-2181.

Rose PD, Phillips TD, Sanderson RD, inventors; Sasol Chemical Industries Limited, assignee. 1995 Jan 3. Solvent extraction. US patent 5,378,369.

Sadler G, Davis J, and Dexman D. 1990. Rapid extraction of lycopene and β-carotene from reconstituted tomato paste and pink grapefruit homogenates. J. Food Sci. 55:1460-1461.

Sander LC, Sharpless KE, Craft NE, and Wise SA. 1994. Development of engineered stationary phases for the separation of carotenoid isomers. Anal. Chem. 66:1667-1674.

Sander LC and Wise SA. 1987. Effect of phase length on column selectivity for the separation of polycyclic aromatic hydrocarbons by reverse-phase liquid chromatography. Anal. Chem. 59:2309-2313.

Schmidt M, Mirza S, Schubert R, Rodicker H, Kattanek S, and Malisz J. 1999. Nanofiltration membranes for separation problems in organic solutions. Chemical Engineering Technology 71(3):199-206.

Schonfelder D and Wilke G, inventors; Veb Filmfabrik Wolfen, assignee. 1971 July 28. Process for the manufacture of dialysis membranes of plasticized regenerated cellulose from vicose. European patent GB 1240560

Schultz B, Paust J, Schneider J, inventors; BASF Aktiengesellschaft, assignee. 1978 Aug 8. Manufacture of symmetrical carotenoids. US patent 4,105,855.

Sharma SK and Le Maguer M. 1996. Lycopene in tomatoes and tomato pulp fractions. Ital. J. Food Sci. 2:107-113.

Sheth J, Qin Y, Sirkar KK, and Baltzis BC. 2002. Nanofiltration-based diafiltration process for solvent exchange in pharmaceutical manufacturing. Journal of Membrane Science 211:251-261.

Shi J and Le Maguer M. 2000. Lycopene in tomatoes: chemical and physical properties affected by food processing. Critical Reviews in Food Science and Nutrition 40(1):1-42.

Shi J, Le Maguer M, Yakuda Y, Liptay A, and Niekamp F. 1999. Lycopene degradation and isomerization in tomato dehydration. Food Research International 32(1):15-21.

Shierle J, Bretzel W, Buhler I, Faccin N, Hess D, Steiner K, and Shuep W. 1997. Content and isomeric ratio of lycopene in food and human blood plasma. Food Chemistry 59(3):459-465.

Snyder LR. 1974. Classification of the solvent properties of common liquids. Journal of Chromatography 92:223-230.

Shukla R. 2000. Production of zein from dry milled corn by solvent extraction and ultrafiltration. Ph.D. dissertation. Urbana-Champaign: University of Illinois. 153 p. Available from Agricultural Bioprocess Laboratory, Food Science and Human Nutrition, University of Illinois.

Shukla R and Cheryan M. 2002. Performance of ultrafiltration membranes in ethanol-water solutions: effect of membrane conditioning. Journal of Membrane Science 198:75-85.

Simpson KL, Lee TC, Rodriguez DB, and Chichester CO.. 1976. Metabolism in senescent and stored tissues.In: Chemistry and Biochemistry of Plant Pigments. Vol.1. 2nd ed. New York: Academic Press. p 779-842.

Stahl W and Sies H. 1996. Perspectives in biochemistry and biophysics. Arch. Biochem. Biophys. 336(1):1-9.

Stahl W and Sies H. 1992. Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. J. Nutr. 122:2161-2166.

Steinmetz KA, Potter JD, and Folson AR.1993. Vegetables, fruit, and lung cancer in the Iowa women's health study. Cancer Res. 53:536-543.

Tajima K and Tominaga S. 1985. Dietary habits and gastrointestinal cancers: a comparative case-control study of stomach and large intestinal cancer in Nagoya, Japan. Jap. J.Cancer Res. 76:705-716.

Taungbodhitham AK, Jones GP, Wahlqvist ML, and Briggs DR. 1998. Evaluation of extraction method for the analysis of carotenoids in fruits and vegetables. Food Chem. 63:557-584.

Tonucci LH, Holden JM, Beecher GR, Khachik F, Davis C, and Mulokozi G. 1995. Carotenoid content of thermally processed tomato-based food products. J. Agric. Food Chem. 43:579-586.

Uragami T, Maekawa K, and Sugihara M. 1978. Studies on syntheses and permeabilities of special polymer membranes 14. Permeation characteristics of modified nylon-12 membranes. Desalination 27(1):9-20.

Van Oss CJ. 1970. Ultrafiltration membranes. In: Perry ES and Van Oss CJ editors. Progress in Separation and Purification. Vol. 3. New York: Wiley-Interscience. p. 97-132.

Van der Bruggen B and Vandecasteele JG. 2002. Fluxes and rejections for nanofiltration with solvent stable polymeric membranes in water, ethanol and n-hexane. Chemical Engineering Science 57:2511-2518.

Wei Y, Zhang T, Xu G, Ito Y. 2001. Application of analytical and preparative high-speed counter-current chromatography for separation of lycopene from crude extract of tomato paste. Journal of Chromatography A 929:169-173.

Weisburger JH 1998. Evaluation of the evidence on the role of tomato products in disease prevention. Proc. Soc. Exp. Biol. Med. 218: 140-143.

Wernick, DL, inventor; 1987. Preparation of cellulose acetate membrane and its use for polar solvent-oil separation. US patent 4,678,555

White LS and Nitsch AR. 2000. Solvent recovery from lube oil filtrates with a polyimide membrane. Journal of Membrane Science 179:267-274.

Whu JA, Baltzis, BC, and Sirkar KK. 2000. Nanofiltration studies of larger organic microsolutes in methanol solutions. Journal of Membrane Science 170:159-172.

Whu JA, Baltzis BC, and Sirkar KK. 1999. Modeling of nanofiltration – assisted organic synthesis. Journal of Membrane Science 163: 319-331.

Wilberg V.C. and Rodriguez-Amaya D.B. 1995. HPLC quantification of major carotenoids of fresh and processed guava, mango, and papaya. Lebensmittel Wiss. Technol. 28:474-480.

Yang, XJ, Livingston AG, and Freitas dos Santos L. 2001. Experimental observations of nanofiltration with organic solvents. Journal of Membrane Science 190:45-55.

Zschocke P, Strathmann H. 1980. Solvent resistant membranes from poly-(p-phenylene-terephthalamide). Desalination 34: 69-75.

Zechmeister L. 1962. Cis-trans Isomeric Carotenoids, Vitamins A, and Arylpolyenes. New York: Academic Press. 251 p.

Zechmeister L and Polgar A. 1944. Cis-trans isomerization and cis-peak effect in the α-carotene set and in some other stereo isomeric sets. J. Am. Chem. Soc. 66:137-144.

Zechmeister L, Le Rosen AL, Went FW, and Pauling L. 1941. Prolycopene, a naturally occurring stereoisomer of lycopene. Proc. Natl. Acad. Sci. 27:468-474.

Zumbrunn A, Uebelhart P, and Eugster CH. 1985. HPLC of carotenes with y-end groups and (Z)-configuration at terminal conjugated double bonds, isolation of (5Z)-lycopene from tomatoes. Helv. Chim. Acta 68:1540-1542.

Zwijnenberg HJ, Krosse AM, Ebert K, Peineman KV, and Cuperus FP 1999. Acetone stable nanofiltration membranes in deacidifying vegetable oil. J. Am. Oil Chem. Soc. 76:83-87.

VITA

Felipe Andres Arana Rodriguez was born in Cali, Colombia on September 23, 1963. He obtained his Bachelor of Science in chemical engineering from Universidad del Valle, Cali, Colombia in 1989. He started his professional career at Lloreda S.A., a vegetable proteins, fats and oils and soaps Colombian company where he held several positions; production engineer, project engineer, and project manager. In January 2001, Felipe Andres started his graduate studies at Texas A&M University and in May 2004 he received his Master of Science in food science and technology.

During his master's program, Mr. Arana gained practical pilot plant experience in membrane technology, centrifugation and spray drying. In 2002 he had the opportunity to work on projects related to purification of nutraceuticals and soy protein isolates. His area of research entails separation and purification of phytochemicals and nutraceuticals.

Mr. Arana has taken undergraduate courses in quality control, unit operations, mass and energy balances, transport phenomena, chemical reactions, and plant design, and graduate-level courses on microbiology of foods, chemistry of foods, food quality, HACCP (certified professional), oilseed protein for foods, oil and fats food products, and statistics in research. During his professional and academic career, he has attended several international conferences, technical plant visits, and short courses which include: Intensive program in functional foods and nutraceuticals, Ghent University, Slovakia, 2003; Institute of Food Technologists Meeting, Chicago, IL, 2003; Practical short course in fats and oils processing and lab analysis, Texas A&M, 2002; Practical short course in membrane technology, Texas A&M, 2001; AOCS Latin American Conference, Cartagena, Colombia, 1999; Visits to extrusion equipment manufacturers: Wenger, Kansas City, KS, 1997; International exposition of packing machinery, INTERPACK, Germany, 1996; Visits to margarine plants in Brazil, Argentina, Switzerland, and Germany, 1993. His professional goal is to obtain a project leader position in the research and development, engineering or manufacturing areas in a challenging environment in the food industry.

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